December 1961

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AMERICAN PHARMACEUTICAL ASSOCIATION

formerly Scientific Edition, Journal of the American Pharmaceutical Association

The *Journal of Pharmaceutical Sciences* considers manuscripts for publication in the following categories:

Review Articles—Summaries covering the more recent advances in an area in which the author has been actively engaged will be published as review articles. Such papers are intended to be critical evaluations of published reports which have previously appeared in the scientific literature.

Research Articles—Reports of original laboratory research in pharmacy will be considered for publication in this section. Pharmacy is defined here in the broadest sense, and includes pharmaceutically related investigations in allied basic disciplines.

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Drug Standards—Reports which include proposed specifications and assay procedures for basic drugs or their dosage forms will be published in this section as they become available. Such reports will generally include discussions of the methods proposed and an explanation of the procedures chosen.

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JOURNAL OF

Pharmaceutical Sciences

December 1961

volume 50 number 12

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The Editor comments

FIFTY YEARS OF SERVICE

This month marks the fiftieth anniversary of the publication of this Association's Journals, and the event is being appropriately commemorated in the "Golden Anniversary," December 1961 issue of the *Journal of the American Pharmaceutical Association*. Although a number of forerunners had been issued earlier, such as the *Proceedings* and the *Bulletin*, it was just fifty years ago that Volume I, Number 1 of the original JOURNAL made its appearance.

In the interim since then, the periodical has undergone a number of major changes, largely resulting from its growth in content, expansion in scope, and increase in circulation. Probably the most notable changes were the division of the JOURNAL into the *Scientific Edition* and the *Practical Pharmacy Edition* in 1940, and subsequent re-titling of the two editions in January of this year. Therefore, although the name of THIS JOURNAL was recently changed, it nevertheless can trace its history and direct continuity back fifty years to that inaugural issue of January 1912.

This half century of service is most significant to the Association and indeed to all American Pharmacy. One of the chief criteria in judging whether a certain activity or pursuit qualifies for consideration as a profession is the existence of an organized literature in that field. This literature must relate to the accomplishments of the members of that group and must include periodicals in which original research can be recorded. In this manner those actively engaged are able to add to the body of knowledge in that discipline, to communicate information to other interested individuals, and to advance the practice of that profession—thereby enhancing its status. Obviously such a literature will be dynamic if it is to fulfill properly its role as part of an active profession.

The publication program supported by the A.PH.A. has certainly been of this nature, and unquestionably the JOURNAL OF PHARMACEUTICAL SCIENCES represents a fundamental asset to pharmacy at a time when it appears particularly important to emphasize pharmacy's basic professional character.

Edward G. Feldmann

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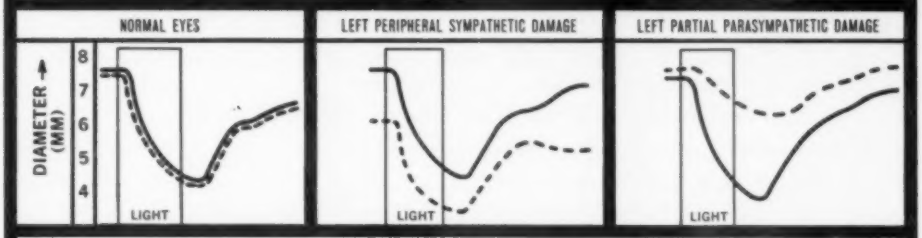

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The next annual meeting of the American Pharmaceutical Association will be held in Las Vegas, Nevada, March 25–30, 1962. Titles and abstracts (not to exceed 200 words) of papers must be submitted to the appropriate Section secretary not later than January 1, 1962, or preferably earlier, in order to assure a place on any Section program. Papers should be sent to the appropriate Section secretary; the names and addresses of these secretaries are listed below.

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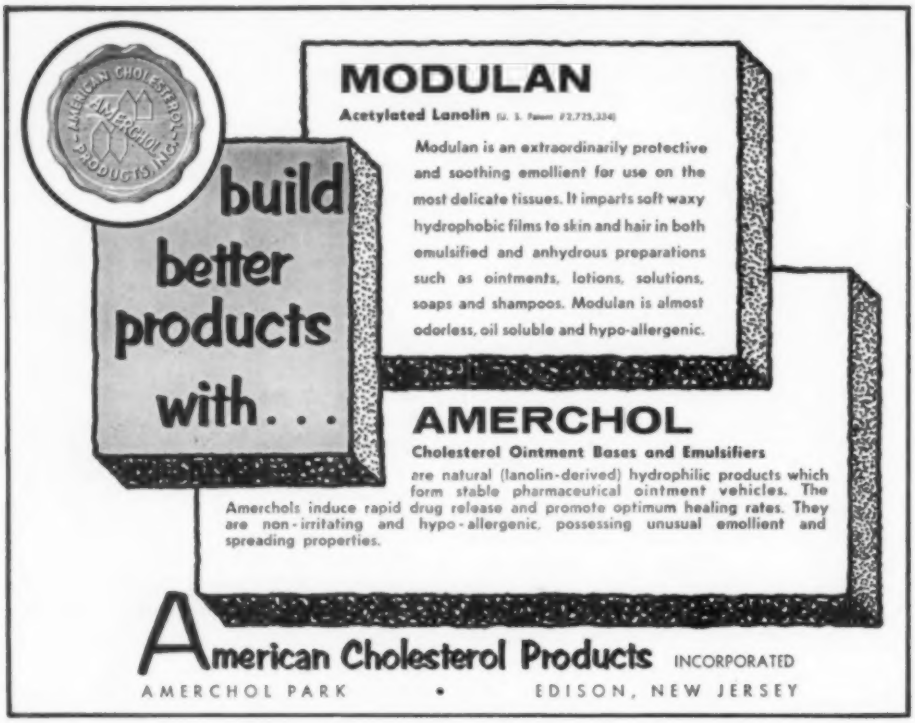
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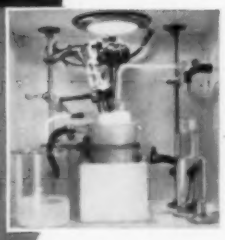

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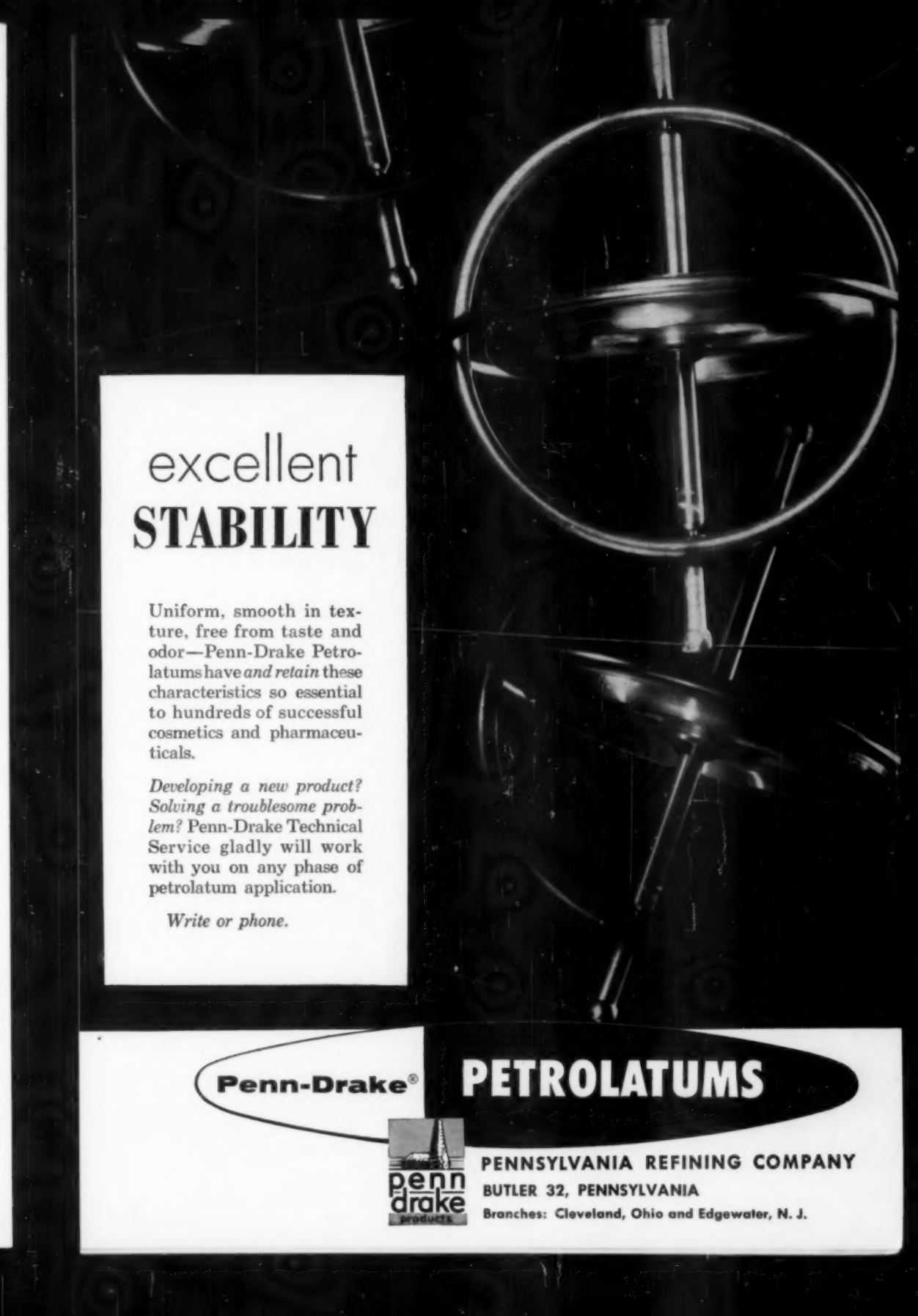

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JOURNAL OF **Pharmaceutical Sciences**

December 1961 volume 50, number 12

Review Article

Influence of Gibberellic Acid on Medicinal Plants

By LEO A. SCIUCHETTI

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INTRODUCTION

THE AMAZING effects of the gibberellins on plant growth has been well established due to the extensive number of investigations reported during the past eight years by plant physiologists, agronomists, botanists, and horticulturists. This group of plant growth-promoting substances has probably stimulated more interest among these workers in a comparatively short time since their potentialities were recognized than did the auxins several decades ago. Well over a thousand publications have appeared describing the effects of these interesting compounds on plant growth. A review of the literature has revealed, unfortunately, a paucity of research regarding the effects of gibberellic acid (G.A.) on the biogenesis of the active constituents of medicinal plants. During the past three years pharmacognosists and allied workers have become interested in this growth stimulator. As a result valuable contributions have been made toward a better understanding of the influence of this chemical upon the growth of medicinal plants and the subsequent formation of alkaloids, glycosides, volatile oils, and other components.

It is not the intent of the author to present a comprehensive review of the effects of G.A. on plant growth. Papers have been cited only as they appeared to be pertinent. Stowe and Yamaki have presented a comprehensive review covering the literature through 1956 (1). A later publication by them covered developments for the following two years (2). Other recent summaries include those by Wittwer and Bukovac (3), Brian (4), and Brian and Grove (5). Stodola has made a most noteworthy contribution to the literature in this area by assembling in one source abstracts of all papers relating to the gibberellins from 1828–1957 (6). Also several summaries and literature abstracts by some manufacturers are available (7–10). In view of the wealth of information already available this discussion will concern itself primarily with the effects of G.A. on germination, growth, and formation of the active constituents of medicinal plants. Likewise, a brief summary concerning the

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After completion of the manuscript, the 1961 edition of "Annual Review of Plant Physiology" was received. The reader is referred to the article by Stuart and Cathey on the applied aspects of the gibberellins (198).

recent developments on the chemistry, effects on cell extension, and division, as well as certain metabolic effects of this growth stimulator will be presented.

HISTORICAL

It has been only during the past seven years that this growth stimulator, which had an oriental beginning 35 years ago, has occupied the attention of many investigators and has opened up exciting new areas of research. The first suggestion of a gibberellin-like effect due to a fungus was a paper which appeared in 1912 by Sawada, a Japanese plant pathologist (11). Sawada believed that the "bakanae" effect was due to stimulation of the plant system by the mycelium found on infected plants (12). Dr. Eiichi Kurosawa, a young graduate of Chiba Horticultural College, came to Formosa in the early 1920's to work with Sawada. His problem was to investigate means of controlling a disease of rice commonly known in his country as the "bakanae" or "foolish seedling disease." This disease is caused by infection of the rice plants by the soil-bearing fungus, *Gibberella fujikuroi*, and causes infected plants to grow unusually tall; later they become weak and eventually die. Kurosawa demonstrated that if he grew this fungus on a nutrient medium for several weeks and then treated healthy rice plants with cell-free filtrates from the growth medium, these seedlings grew more rapidly than controls without any infection by the fungus. He concluded that some metabolic product of the fungus had accumulated in the culture media and that it had plant growth-promoting properties (13). The first photograph found in the literature which illustrated the stimulatory effect of the fungus secretion appeared in Kurosawa's paper (11). Thus, Kurosawa is given credit for pioneering the work with the gibberellins.

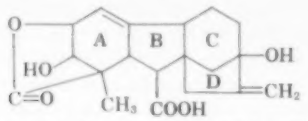
Kurosawa and others attempted, without success, to isolate the active material. It was not until 1935, however, when Yabuta of the University of Tokyo isolated the crystalline active material and named it gibberellin (14). Three years later Yabuta and Sumiki characterized gibberellin (15) and since then these workers and their colleagues have published many papers on the bakanae substances and related items. Due to the war, publications of their investigations failed to reach American and European workers. The development of techniques for the mass culture of fungi for antibiotic production was applied to the production of gibberellin by occidental workers after the abstracts of the Japanese workers became available in 1950 (16–18).

The first work on gibberellin outside of the orient was started about ten years ago, apparently independently, by a group at the United States Department of Agriculture under Stodola and another group at Imperial Chemical Industries in Britain under Brian, Burrow, and others (1). As a result of the findings of these early workers (16–18), relatively large quantities of gibberellins for experimental purposes became available. Many pharmaceutical manufacturers have adapted their antibiotic equipment to the production of G.A. and other gibberellins. In 1957 a gram of G.A. cost about ten dollars, whereas today the same quantity can be purchased for about two dollars (19). Thus, cost is not a limiting factor in the commercial use of the growth stimulator since only micro amounts are needed to elucidate a response in plants.

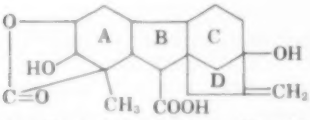
CHEMISTRY OF THE GIBBERELLINS

Gibberellin is the generic name of several closely related chemical substances which exert profound effects on plant behavior. Presently, seven gibberellins have been characterized and identified. These are known as gibberellins A_1, A_2, A_3, A_4, A_5, A_7, and A_9. Gibberellin A_3 proved to be identical with a substance, reported as gibberellin X, and is now known as gibberellic acid (G.A.). G.A. has been employed by most investigators since it is believed to be the most active of the gibberellins and a relatively pure form is available.

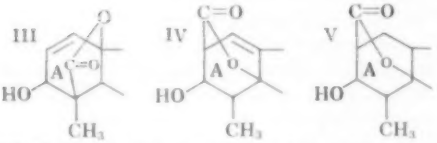
The chemistry of the gibberellins derived from culture filtrates of the fungus *Gibberella fujikuroi* has been extensively investigated by two groups of chemists, one at the College of Agriculture of the University of Tokyo and the other at the Akers Research Laboratories of the Imperial Chemical Industries, Ltd., in England (20). Grove, *et al.*, of the Akers group proposed structure I for gibberellic acid (21) and II for gibberellin A_1 (22). Cross, Grove, and collaborators later revised the proposed structure of G.A. so that the structure of ring A corresponds to III (20). Sumiki and his group at the University of Tokyo have proposed the structure for G.A. and A_1 as in I and II except for the position of the lactone ring in A as depicted in IV and V (23–25). The Japanese workers have also proposed structures for gibberellin A_2 and A_4 which they isolated from fungal filtrates. They believe that the ring structure is the same as in A_1 (V) but are modified in the C—D rings as in VI and VII. The B ring in both cases is identical to that in I.



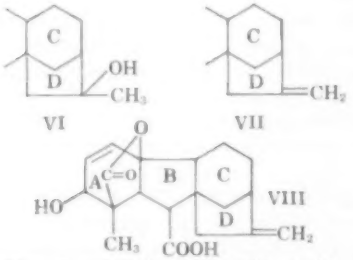
I. Gibberellic Acid, G. A. (GA-3, Gibberellin X, A_3)



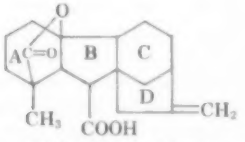
II. Gibberellin A_1 (Dihydrogibberellic acid, GA-1)



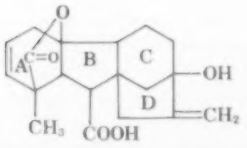
III. Revised ring for G.A. (Cross, *et al.*). IV. Ring A in G.A. (Sumiki, *et al.*) V. Ring A in A_1. (Sumiki, *et al.*)



VI. Ring C and D in A_2 (Sumiki, *et al.*). VII. Ring C and D in A_4. (Sumiki, *et al.*). VIII. Gibberellin A_7. (Cross, *et al.*)



IX. Gibberellin A_9 (Cross, *et al.*)



X. Gibberellin A_8 (MacMillan, *et al.*)

Fig. 1.—Chemical structures of the gibberellins.

Cross, *et al.*, recently discovered two additional gibberellins, A_7 and A_9, which are produced by *Gibberella fujikuroi* (26, 27). Their proposed structures are depicted in VIII and IX. Also West (20) has reported that MacMillan, *et al.*, have isolated A_8 from *Phaseolus multiflorus* and assigned a structure to it as in X. He believes it is identical to his bean factor II. Thus, it can be seen that structurally the gibberellins are closely related. For example, G.A. differs from A_1 only in the presence of a double bond in ring A; from A_7, in the presence of a hydroxyl group in ring C. On the other hand, gibberellins A_2, A_4, and A_9 differ from A_1 only in a difference in the substituents in rings C—D (Fig. 1).

The evidence is conclusive that substances with biological properties similar to the fungal gibberellins are present in flowering plant tissues. The first description of the occurrence of a gibberellin-like substance in higher plants was reported by Mitchell, *et al.*, in 1951 (28). West and Phinney (29) isolated bean factor I and bean factor II from immature bean seeds and believe that bean factor I is identical to gibberelin A_1, but West (20) proposed a structure different from the known gibberellins for bean factor II. A comparison of the infrared spectrum of the latter with that of A_8 of MacMillan showed they were identical. MacMillan and Suter isolated A_1 from immature bean seed (30), and Sumiki and Kawarada isolated A_1 from water sprouts of the mandarin orange (31). A number of other reports relating to the isolation of gibberellin-like substances from plants have been published (32–37). However, the substances were not isolated in a sufficiently pure state to permit a useful determination of the physical and chemical properties of them.

Extensive greenhouse and field investigations have been conducted and the results summarized for a wide variety of plant responses produced by commercial gibberellin preparations composed largely of G.A. (about 90%) or undetermined mixtures of A_1 and A_2 (1–4). Bukovac and Wittwer studied the comparative biological activities of the various gibberellins and two methyl-ester derivatives on promotion of stem elongation in dill (38). They found the following relationships: $A_3 > A_1 \backsimeq A_4 >$ methyl ester $A_1 \backsimeq$ methyl ester A_3. This indicates that esterification of the carboxyl group reduces the biological activity of G.A. (A_3). It has also been shown that the complex lactone ring is essential for activity since potency is lost when it is opened and gibberellenic acid is formed (39, 40).

Bukovac and Wittwer in a later publication (41) compared the biological activities of gibberellins A_1, A_2, A_3, and A_4 and their derivatives in intact plants such as beans, celery, cucumbers, lettuce, tomato, and Weigela plants. Among the areas of plant behavior studied were flowering, fruit growth, vegetative extension, and dormancy. The biological activities of the four gibberellins and their derivatives varied with the assay, site of treatment, and the amount applied. It was interesting to note that they found that gibberellin A_4 had a markedly greater effect on the stem elongation of cucumber. This has also been confirmed by Halevy and Cathey, who found that A_4 was significantly more active than A_1, A_2, or A_3 in promoting hypocotyl elongation of seedlings grown in light (42), and by Lockhart and Deal in the same plant (43). Likewise, Brian and Hemming reported that gibberellin A_1 and A_9 were considerably more active in promoting hypocotyl growth in cucumbers than G.A. (44). This indicates that plants are selective in their response to the various gibberellins. Further, this specificity of certain plants to definite responses by a selected gibberellin could be utilized as a bioassay for differentiation between the naturally occurring gibberellins in plants. Halevy and Cathey in their study with cucumber seedlings also found that the potassium, sodium, and zinc salts of G.A. (A_3) were as active as the free acid and that the butyl cellosolve ester was almost as active as the acid. However, the methyl, butyl, and octyl esters were relatively inactive.

Since G.A. (gibberellin A_3) is readily available and has been utilized to the greatest extent, a few pertinent facts are in order. The total content of G.A. varies somewhat according to the commercial source.[1] Our supply contains 88.9% of gibberellic acid. It is prepared by submerged synthetic cultures of *Gibberella fujikuroi*, with the yield being about 10 mg./L. of medium. It contains about 4.7% moisture and 1.5% gibberellenic acid (a hydrolytic product of G.A.). Its specific rotation is +75.2; m.p. about 240°. Pure gibberellic acid has the molecular formula $C_{19}H_{22}O_6$ and a molecular weight of 346.4. Six milligrams of pure G.A. dissolves in 1 ml. of water at 25°. It is readily soluble in cold methanol, ethanol, acetone, and isopropanol, and less readily soluble in ethyl acetate, ether, and petroleum ether. Dry G.A. is stable at room temperature. Aqueous solutions lose potency at room temperature (25°). To avoid excessive loss of potency, aqueous solutions should be prepared fresh before applying and never kept for more than a week even under refrigeration. Preparation of a stock solution in absolute alcohol and storing in a refrigerator offers a method of convenience and stability to the investigator. Dilution of aliquots from the stock solution with water can then be made as needed.

[1] We are indebted to Dr. Edwin F. Alder, Agricultural Research Center, Eli Lilly and Co., Greenfield, Ind., for the generous supply of G.A. employed in our studies.

CELL EXTENSION AND DIVISION

The most characteristic and consistent vegetative response of plants to G.A. is an increase in internode length. This is especially true in cereals. Increased cell length accounts for all or most of this internode or cereal leaf elongation (4). Kato (45) and Brian, *et al.* (46), believe that cell extension is implicated. It appears that cell elongation rather than cell division is the primary effect in rice and other cereals. However, recent evidence indicates that cell division is induced in many other plants. Lang in 1956 suggested that when gibberellin was applied to nonvernalized biennial *Hyoscyamus niger*, the formation of an elongate stem was due to an increase in the number of cells (47). A year later Lang and Sachs presented direct evidence that gibberellin caused an increase in cell division in biennial *H. niger* (48). Others have indicated that the increased stem elongation of *Phaseolus* from gibberellin treatment involves a considerable increase in cell multiplication as well as extension (49, 50).

The most comprehensive studies concerning the effect of G.A. in the plant cell are the recent reports of Sachs and co-workers. They found in the course of their studies on the cytological and histological effects of G.A. that stem formation in rosette plants is due not to an increase in the cell division activity of the existing apical meristem, but to the appearance of a new zone of very active cell division immediately below this meristem (51–53). It was suggested that gibberellins or gibberellin-like, native growth substances were factors limiting the subapical meristematic activity in rosette plants. Thus, the transition from the rosette habit of growth to stem elongation is the result of cell division in the subapical region of the shoot and this process is controlled by gibberellin. Sachs, *et al.* (54), further observed in a study of two varieties of a caulescent plant, *Chrysanthemum morifolium*, that Amo-1618, (4-hydroxy-5-isopropyl-2 methylphenyl) trimethylammonium chloride, completely inhibited subapical mersitematic activity. G.A., applied either simultaneously or following the Amo-1618 treatment, completely prevented or

reversed the effect of Amo-1618, making the plants retain or resume normal growth habit. Either compound had very little influence upon the activity of the apical meristem. They concluded that gibberellins, or native, gibberellin-like substances appear to regulate the activity of the subapical meristem and thus play an important role in shoot development. On the other hand, Amo-1618 and related compounds appear to exert their dwarfing effects in plants by acting as gibberellin antagonists.

Sachs and Lang in a recent publication (55) report on the influence of G.A., Amo-1618, and maleic hydrazide on shoot histogenesis and the subapical meristem. Seven different plants were employed. They concluded that G.A. caused stem elongation in rosette plants by stimulating mitotic activity immediately below the apical (pro) meristem. The cells produced in this zone, after elongation, constitute the tissues of the mature, elongate stem. In caulescent plants a subapical zone of mitotic activity was observed as much as 2 cm. in length, similar in activity to that in rosette plants receiving prolonged treatment with G.A. By treatment with Amo-1618, subapical (but not apical) cell divisions were considerably reduced and, soon after, shoot elongation ceased. G.A. prevented and reversed the inhibition of subapical cell division induced by Amo-1618. Thus, in caulescent as well as in rosette plants, G.A. plays an important role in regulating subapical mitotic division, thereby controlling shoot elongation (55).

It would appear from the reports in the literature on the action of G.A. in the cell that both cell extension and cell division play an important role in G.A.-induced growth. The recent work of Sachs and co-workers indicate that cell division is implicated to a greater extent than heretofore believed. This is one of but many examples (1–5) where investigations with G.A. have given a better understanding of the many complex biological processes involved in plant growth. Steward and Schantz (56) state that the twin aspects of growth, cell multiplication and cell enlargement, are commonly treated separately, as though they were subject to distinct stimuli and controlling factors. However, cells divide first and then they enlarge. These two growth processes frequently merge smoothly, the one into the other, and indeed often overlap (56).

DORMANCY AND SEED GERMINATION

The familiar winter dormancy of deciduous trees and shrubs is a photoperiodic phenomenon induced by the short days of winter. Growth will only start again after exposure to an increased day length and/or exposure to low temperatures. Treatment of dormant twigs of beech and ash with G.A. induced resumption of growth without exposure to the normal stimuli (57). Dormancy of resting buds of *Fagus sylvatica* (58), summer dormancy, arrested fruit growth, of Marglobe tomatoes (59), and epicotyl dormancy of tree peonies (60, 61) have been broken by G.A. treatment. The influence of G.A. on the photoperiodically-controlled dormancy of woody plants has been investigated (62–67). Favorable responses were reported in the Elberta peach, *Acer*, *Camellia*, and some species of *Pinus*, whereas no effects were noted in *Pinus coulteri* and *Pseudotsuga macrocarpa*. On the other hand, it is interesting to note that application of gibberellin in the fall to *Vitus vinifera* prolonged the dormancy of the shoots of this woody plant the following spring (68). Brian, *et al.*, reported that application of G.A. in late summer and autumn delayed leaf fall in several woody species (69). Further observations on these plants the following spring showed that dormancy in many of them was prolonged (70). Properly chosen treatments with gibberellin in the autumn might usefully be employed to delay flowering in the following spring, in areas where frost damage is a hazard of fruit cultivation (68, 70).

The dormancy of potato tubers is broken by treatment with G.A. This type of dormancy is not related to photoperiod or any requirement for vernalization (cold treatment), but disappears gradually following the harvest of the tubers. A condition of physiological rest for a period of from 6 to 12 weeks after harvest appears to be necessary before sprouting occurs. Dormancy of potatoes and induced sprouting have been reported by several workers (71–74). Earlier and more uniform sprouting which results in a more rapid maturity of the crop has resulted from treatment of both dormant and nondormant tubers. Chao has broken the dormancy of artichoke tubers and Irish potatoes by a gibberellin treatment (75).

Treatment of seeds with G.A. has, in many cases, overcome seed dormancy and has promoted an earlier and more uniform emergence of the seedlings. The germination process is a complex series of biochemical and physiological changes involving the initiation of growth and mobilization of reserve materials within the seed to be used by the embryo during growth. The process is controlled by a system of interlocking reactions. These must at all times be kept in balance. The following sequence of reactions probably

occur during germination: imbibition of water, enzymatic and respiratory activity, digestion, translocation, assimilation, and growth. For a more complete discussion of this process see Toole, *et al.* (76), and Koller (77).

The occurrence of gibberellins in seeds is now well established (4, 78–80). It appears that gibberellins and auxins are essential for the proper maturation of seeds. Edwards, in 1940, made the first suggestion of a stimulating effect of gibberellin in the germination of corn seeds (81). Kahn reported that gibberellin causes dark germination of lettuce seeds that would not germinate in the absence of a promoter because of a relatively low supra-optimal germination temperature; inhibition produced by far red light; dark-osmotic inhibition; and inhibition induced by a high temperature pretreatment (82). He also concluded that gibberellin is capable of replacing red light in every situation examined in which light promotes germination of lettuce seeds. The breaking of lettuce seed dormancy has also been reported by others (83–85). Further, Skinner, *et al.*, have demonstrated that a combination of gibberellin and 6-(substituted) purines act synergistically in stimulating the rate of germination of lettuce and carpet grass seeds (86). Mayer has reported that G.A. reversed the inhibition of lettuce seeds by coumarin (87). Other plants demonstrating an increased germination rate from gibberellin seed treatment include: beans and peas (88, 89); corn, beets, and tomatoes (89); papaya (90); sweet cherry (91); Kalanchoe (92); saguaro cactus (93); wild oats (94); eggplant, spinach, and carrots (95); sugar cane (96); wheat (97); and creeping red fescue (98).

Perusal of the literature revealed several interesting aspects regarding germination and dormancy. The stunting of maize seedlings by radiation of the seeds was reversed by G.A. treatment (99). Also, dormancy of potato tubers induced by irradiation was reversed by G.A. (100). The authors suggested that irradiation impaired a possible endogenous gibberellin-synthesizing system in the potato and that the effects of this damage was counterbalanced by exogenous G.A. Haber and Luippold irradiated wheat grains with a large enough dosage of gamma-rays to abolish all mitotic division and found that G.A. stimulated growth of this grain in the complete absence of cell division. They concluded that G.A. stimulated growth of these irradiated plants by cell expansion alone (101). Gibberellin treatment of cotton seeds (102) and Ladino white clover seeds (103) did not reduce preemergence or postemergence damping off. Actually, damping off was increased by the treatment in the latter. In combination with G.A., urea-formaldehyde and Vapam (Vapam is sodium N-methyldithiocarbamate dihydrate) 45 as drenches, and Orthocide 75 (75% captan) incorporated into a talc seed coating, gave significant control of bean root rot (104). Seeds from flowers, induced by gibberellin treatment of sugi, *Cryptomeria japonica*, germinated as well as those from untreated plants (105). Work in our laboratory has indicated that seeds from stramonium plants treated the previous year with G.A. germinated more rapidly and a more uniform stand was obtained than from seed obtained from untreated plants (106).

On the other hand, unfavorable responses have been reported on the germination of seed from G.A. treatment. Germination of sugar beet seeds has not been affected (107–109). Mes disputed the work of others in that she found that gibberellin did not eliminate the chilling requirement for peach seeds (110). The growth of plants from unchilled seeds was stunted by G.A. although germination was increased. No improvement was noted in the germination of *Pinus strobus* and *Picer abies* (111). G.A. treatment of the pollen (112) or seed (113) of Douglas fir did not affect the total germination. Likewise, unfavorable responses were observed in other plants (114–116). Corns reported that winter survival and vegetative yield of wheat was adversely affected by gibberellin treatment (117). Howell, *et al.*, studied the response of soybeans to seed treatment with gibberellin under simulated commercial conditions and found that stands and yields were reduced (118). Maturity as well as oil and protein content of the seed, however, were not affected consistently by the treatment.

From a review of the literature, apparently the only work conducted on the effects of G.A. on the germination of seeds of medicinal plants has been that at Oregon State University. Seeds from *Atropa belladonna*, *Hyosyamus niger*, *H. albus*, and *Datura stramonium* were employed (119). These experiments have shown that, in general, the rate of germination as well as the total emergence was significantly increased. Three groups of seeds were soaked for 18 to 24 hours in solutions containing either 50, 100, or 1000 p.p.m. of G.A. Observations from the growth of such seedlings indicated that considerable alteration in their normal growth and alkaloid patterns were induced with the higher concentration of G.A.

In another experiment conducted in the spring of 1959, 800 "uniform" seeds obtained from *Datura stramonium* harvested in 1958 were divided into two groups of 400 each (119). The first group was treated with 50 p.p.m. of G.A. for 18 hours prior to planting; the second was not treated. On April 21, seeds were planted in flats containing soil and germinated under greenhouse conditions. Significant increases were noted in the rate of germination of the seeds treated with G.A. Three weeks after planting, the germination rate was increased about 100% by the treatment. Seedlings from treated seeds were taller, thinner, and paler in appearance than controls. Also, they demonstrated a higher incidence of damping off and root rot. Concurrently, a similar experiment was conducted with seeds of *Hyoscyamus albus* (119). In this case, the germination rate was increased about fivefold.

Scott and Sciuchetti employed various treatments, including G.A., on the seeds of *Atropa belladonna* and studied the effects of the treatment on the germination and subsequent growth and alkaloid formation in the plant (120). The seeds treated with G.A. were the first to germinate and attained the highest germination rate throughout the 5-week observation period. The per cent increases in germination over controls were about 250 and 40% at the third and fifth weeks, respectively. The seedlings emerging were healthy and sustained satisfactory growth thereafter. They were slightly taller than controls and closely resembled the latter in habit. Fresh and dry weight data, in general, indicated a favorable response in the treated group during a 4-week observation period. The total alkaloidal content was increased at the first and second harvest but was reduced slightly at the terminal harvest. The concentration of alkaloids in the plant parts fluctuated only slightly compared with controls throughout the observation period. The authors concluded that the overall response of belladonna to a gibberellin seed treatment was favorable since the rate of germination and total germination was greatly increased, whereas changes in alkaloid content were slight.

A similar experiment was conducted on the seeds of *Datura stramonium* (121). A threefold increase in germination was noted in the treated seeds. Throughout the observation period the habit of these plants closely resembled the controls except that they were slightly shorter than plants grown from untreated seeds. It was noted from dry weight data that during the early stages of growth the treatment induced a deleterious effect. However, no significant change was observed in weights at the final harvest. The concentration of alkaloids in the plant organs was not altered markedly. Due to the decreased growth the total alkaloidal content of the plant at the first two harvests was considerably reduced in the treated group. However, no significant difference was observed at the final harvest. The seed treatment was considered beneficial since a greater and more uniform emergence of seedlings was obtained while the total alkaloid content per plant was reduced only slightly at the final harvest.

The beneficial response in germination by the seeds of many plants to gibberellin treatment has a number of practical applications. In the setup of experiments for teaching or research in pharmacognosy and allied areas, the growth phases of an experiment is usually conducted during the spring term. This necessitates that the seeds be planted in flats in the greenhouse in winter. We have found that the germination rate of many seeds from medicinal plants is slow and erratic. A uniform stand is difficult to obtain. Loss of seedlings from damping off is usually high. Gibberellin treatment of the seeds appears to be the solution to the problem since it apparently substitutes for some specific environmental condition and breaks seed dormancy. Species differ in their response to G.A. Further, it should be ascertained whether any changes in plant composition result from the treatments. Thus, a fertile area of research is opened up. Horticulturists and agronomists have taken advantage of this speed-up of the germination process where it has been demonstrated. Further, a more rapid emergence of young seedlings should give them a better chance for survival. Finally, the eradication of weeds could be facilitated if seed treatment with G.A. would result in a greater potential weed population being available at one time for application of the proper herbicide.

VOLATILE OIL-CONTAINING PLANTS

Until the past 3 years, most of the research on plants producing volatile oils has been concerned with growth responses of G.A. For example, Stodola (6) in his excellent publication lists four references for mustard (*Brassica juncea*), three for rape seed (*Brassica napus*), and two each for citron (*Citrus medica*) and orange. None of the abstracts listed indicate work for analyses of volatile oils or active components, but describe growth responses from the use of G.A. Scurfield and Moore (122) reported that various

species of *Eucalyptus* grew taller and produced weaker stems from a G.A. treatment. Total weight of treated plants was not significantly affected. Shimada conducted a study on fennel, *Foeniculum vulgare*, to determine whether G.A. would replace the long-day treatment to induce flowering of this long-day plant (123). Gibberellin treatments, instituted on the same day in which the photoperiod was switched from long day to short day, stimulated elongation of internodes of the main shoot, not only in the plants induced near the threshold for successive elongation by 12 long-day treatments, but also in the plants maintained photoperiodically under rosette growth conditions by continuous short-day treatments. G.A. sprayings did not cause differentiation of the flower in either of the two groups (123).

Although hops, *Humulus lupulus*, is now used primarily in the brewing industry, it is classified in this group of medicinal plants. Nash sprayed hops with the potassium salt of G.A. when the stigmas of the female flowers were one-eighth inch or greater in length and observed an initial paleness in the leaves (124). Maturity of the cones was advanced about 10 days and at harvest time the developed cones were at a more uniform stage of development than controls. However, the cones developed from treated burrs were more subject to wind burn. About a 40% increase in yield was obtained. Interestingly, though, the alpha-resin content of the treated hops was 1.8% compared with 10.16% for the controls. Commercial hops are prized for a high resin content. This most important component from a brewer's point of view was greatly decreased by the treatment. Further work on the estimation of volatile oils indicated there was a difference in this component between treated and normal hops.

Several intensive studies have been conducted on the mints. Gonzalez and Gjerstad (125) treated spearmint (*Mentha spicata*) twice with foliar sprays of G.A. in a concentration of 50 p.p.m. and reported a 258% increase in linear growth. The treated plants became so weak that it was necessary to support them in order to keep them upright. Considerable changes in the shape of the leaves were noted and a four- to sixfold increase in the diameter of the stems of the treated plants was observed. A histological study of the stems indicated that the pith cells were smaller and more numerous in the treated plants and that the xylem area was larger and the ribs were missing. The dry weight of the treated plants was considerably higher than controls. Analyses disclosed that the treatment with G.A. induced decreases in the water content, the per cent of total chlorophyll, the concentration of volatile oil, but, no significant change in carvone content or refractive indexes of the oil were noted. Ogzewalla treated peppermint, *Mentha piperita*, with weekly sprays of 10 and 100 p.p.m. of G.A. and noted characteristic gibberellin effects in the treated plants (126). Fresh weight yield was significantly reduced but not dry weight yield. He concluded from observations of histological sections from stems of the treated plants that cell elongation primarily accounted for the change in internode length. There was no significant increase in the number of cells in the longitudinal direction in the stems while a decreased number of cells in all tissues were noted in the stem cross section. Total oil production was reduced and slight changes were noted in the chemical and physical constants of the volatile oil. It was concluded that either the concentration of G.A. employed was too high or the frequency of treatment was too great to obtain optimum oil production.

Gjerstad also induced metabolic and morphological changes in peppermint by spraying the plants at irregular intervals with a 50 p.p.m. solution of G.A. (127). The following statistically significant differences were noted in the treated plants: a 33.5% increase in cauline length, a 28% increase in chlorophyll concentration, a 52.4% reduction in volatile oil yield, and a higher concentration of glucose and sucrose. No significant differences were noted in water content, percentage of dry matter, and total menthol content. No qualitative differences were observed in respect to carbohydrates, amino acids, or Krebs' cycle acids. The gibberellin treatment induced a spindly growth which resulted in smaller leaves and a reduced number of foliar glandular hairs. Histological sections showed that stems had three times as broad a xylem region and the cells' walls of the ribs were thicker and stained less intensely with hematoxylon. The significant decrease in volatile oil content was attributed to fewer glandular hairs, smaller leaves, and an increased metabolism. On the basis of this work, as well as on unpublished work on several other medicinal plants, the author concluded that G.A. is of doubtful value in drug plant cultivation (127).

Your reviewer takes exception to this viewpoint inasmuch as an insufficient amount of research has been conducted in this area to offer a definite conclusion. Many factors have to be determined whether a favorable response of growth and/or

volatile oil synthesis can be obtained. Some of the factors to be considered are: the proper stage of development of a plant both at the time of treatment and time of harvest when beneficial effects can be expected; the concentration of gibberellin to apply which can only be determined by administration of quantitative amounts and not by a foliar spray treatment; the frequency of treatment; environmental conditions at the time of treatment including use of fertilizers; and generic differences. It must be admitted, however, that the three investigations with mint (125–127) did not prove successful in increasing volatile oil production under the conditions of the experiments.

GLYCOSIDE-PRODUCING PLANTS

Most of the investigations on glycoside-producing plants have been performed on *Digitalis purpurea* and *D. lanata*. Blaney, in 1956, induced the biennial foxglove (*D. purpurea*) to flower the first year by daily applications for 30 days of 2 mcg. of G.A. (128). Foxglove after sufficient growth requires a cold-treatment before it flowers. G.A. in this case substituted for the cold requirement. This has also been reported for *D. purpurea* by others (3, 129, 130). Sayed and Beal (131) treated *Digitalis purpurea* twice weekly by spraying with 100 p.p.m. of G.A. for 60 days. Harvesting was done at approximately weekly intervals. The leaves of treated plants developed longer petioles and less surface area than controls; became more linear and almost lanceolate, the dry weight was markedly decreased. Histologically, no difference was observed but treated leaves had a higher palisade ratio and a lower vein-islet number. Some of the treated plants developed stems. An increase in the per cent of glycosides and total sugars was observed in the treated plants, but there was no significant difference in digitoxose per cent. This suggested that the increase in glycoside per cent may have been due to a higher aglycone per cent rather than digitoxose. They also suggested that the seeming increase in number of palisade cells might explain the increase in per cent of carbohydrate since this would mean a larger number of photosynthetic cells per unit area. The total chlorophyll (a and b) content increased. There was an initial increase in crude fiber content of the treated plants. At maturity, however, both treated and controls demonstrated about the same content.

Burton and Sciuchetti treated *D. lanata* with 10 and 50 mcg. of G.A. from mid-May to mid-November in 1958 (132). The plants were 3-week old seedlings when treatment was started and were grown in soil in pots under greenhouse conditions. Typical gibberellin effects were noted in the treated plants such as narrower, longer, and slightly chlorotic leaves. Height measurements which were taken weekly indicated that significant increases were induced by the treatments. At harvest time the plants treated with 10 mcg. of G.A. were about 87% taller than controls and those treated with 50 mcg. were about 63% taller. The form of plant growth was markedly altered and most of the treated plants had bolted. The "bolting effect" was more pronounced with the stronger treatment. Fresh and dry weight data indicated that considerable increases in fresh and dry weights were induced by G.A. Root weight, however, was reduced considerably by the stronger treatment.

The same workers in a later investigation with *D. lanata*, but employing volunteer seedlings, grew the plants in an outdoor shaded garden plot (133). Weekly treatments with 10 and 50 mcg. of G.A. per plant were instituted on July 7 and harvests made of the material at 4-week intervals during a 16-week observation period. Growth responses were similar to that in the previous experiment (132). The treated plants began bolting at the twelfth week. Definite stems were observed at the final harvest while the controls remained acaulescent. G.A. had apparently replaced the cold treatment necessary to induce stem formation in digitalis plants. Heights of the treated plants were significantly increased with the peak (about 60% above controls) noted at the eighth week. Both treatments induced an increase in the dry weights of the shoots with the maximal effect being at the eighth week. Only in the 50-mcg. series was the dry weights of the roots altered, in which case, a significant reduction in root growth was noted. The total glycoside content per shoot of the treated plants demonstrated significant increases during the first 8 weeks. At the final harvest, however, the 50-mcg. series had about half of the total glycoside content of controls whereas the 10-mcg. series demonstrated about a 30% increase. In general, the treatments resulted in an increased level of total and reducing sugars. The concentration of starch was somewhat lower at the eighth and twelfth week, but, a slight increase was noted at the terminal harvest. It was noted that the higher dosage level appeared to be toxic after the eighth week, while the lower dose induced a beneficial response. The authors concluded that the effect on glycoside production

appeared to correlate more closely with the responses on growth than with the effect on carbohydrate fractions.

Several workers (134–136) have reported regarding the growth effects of gibberellin on buckwheat (*Fagopyrum esculentum*). Sayed and Beal studied the influence of G.A. on the growth of the plant and the biosynthesis of rutin (137). Increases were reported in the linear growth, dry weight of stems and leaves, number of leaves per plant, total sugar per cent, crude fiber per cent at early stages, and total chlorophyll per cent. Decreases were noted in rutin and rhamnose per cents. Even though the glycoside (rutin) percentage was relatively lower in treated plants than controls, the relative increase in the dry weight and number of leaves in treated plants resulted in a higher rutin content per treated plant. The authors suggested that increased cell division might account for the increase in stem weight, cellulose content, and plant maturity. Several possible effects of G.A. on glycoside biosynthesis were postulated.

ALKALOID-PRODUCING PLANTS

Among the medicinal plants the most intensive investigations have been done with those producing alkaloids. Thus far, the members of the *Solanaceae* have received the most attention. Henbane (*Hyoscyamus niger*, biennial and annual strains) has received the attention of early investigators. Curry and Wassink (138) reported that G.A. substituted for the long-day requirements necessary for the flowering of annual *Hyoscyamus niger*. Biennial *Hyoscyamus* requires a specific low temperature for a critical period followed by exposure to long days before the plant flowers. Lang (47, 139–141) made the important discovery that G.A. substituted for the cold (vernalization) treatment needed by the plant to flower. Lang's results have since been generally confirmed with other cold-requiring long-day plants. Sachs and Lang (48) reported that an increase in cell division was induced in less than 24 hours in *Hyoscyamus* by G.A. (see previous discussion on cell division). The first work reporting the effect of G.A. on the alkaloid formation in *Hyoscyamus niger* was that by Masuda and Hamor (142) in 1959. G.A. was applied in daily 5-mcg. doses by injection. They reported considerable increase in total dry weight in the treated plants due to an increase in stem weight, but the weight of the leaves decreased slightly. The total alkaloidal content of the overground portion of the plant was somewhat less than that of the untreated plants. The concentration of alkaloids in the leaves was markedly reduced in the treated plants. Significant increases in height were noted.

Belladonna and jimson weed have been studied to a considerable extent regarding the effects of G.A. on the growth and alkaloid biogenesis of the plants. Smith and Sciuchetti, in 1957, applied G.A. in concentrations of 100 and 1,000 p.p.m. as an aqueous spray three successive times at 10-day intervals to the leaves and tops of *Atropa belladonna* (143). Among the specific effects reported for the treated plants were: increased stem elongation which resulted in a two-to-threefold increase in height; a spindly and vine-like type of growth, leaves which appeared slightly chlorotic and were narrower and thinner; more rapid onset of flowering; significant increases in stem dry weights, but significant decreases in leaves, tops, and root dry weights. There were no significant changes in total dry weights.

Of more importance from a commercial aspect was the profound effect that G.A. had on alkaloid formation and accumulation in the plant. It was found that the concentration of alkaloids in the various morphological parts of the treated plants ranged from 43 to 84% that of the controls. Of the organs, the stems showed the greatest decreases at each concentration of G.A. It was also found that the total alkaloid content of belladonna treated with G.A. was about one-half that of the untreated plants.

In a continuation of this study, treated and untreated belladonna were allowed to overwinter and it was reported that the second-year crop of the previously treated plants did not demonstrate the "gibberellin effects" noted in the first-year growth following treatment (144). In some instances reverse effects were noted. The plants previously treated with 100 p.p.m. of G.A. generally closely resembled the controls in their growth and alkaloid patterns. However, plants which received the higher concentrations of G.A. the previous year were markedly affected. This treatment induced about a 50% decrease in total dry weight and approximately a 15% decrease in height. The concentration of alkaloids in the leaves and roots was not significantly altered, whereas, that in the stems was increased about 44%. The total alkaloid content of this group was about 56% that of the controls. It was concluded that the higher concentration of G.A. (1,000 p.p.m.) had a toxic effect on the plant.

In a later investigation, Brummett and

Sciuchetti treated belladonna with a single 100-mcg. dose of G.A. and observed the growth and alkaloid patterns over a 6-week period (145). Characteristic gibberellin effects were induced. Interesting differences in fresh and dry weights were induced in belladonna at different stages of maturity. Younger seedlings indicated significantly decreased growth, whereas, older plants demonstrated significant increases. Height measurements indicated that the latter reacted more rapidly and for a longer period of time than younger plants. Also, height increases were not as great as that induced by a spray-treatment (143). Reduced concentration of alkaloids was noted in the treated plants of both age groups (145). The decreases were greater in the younger plants. When compared with controls at the sixth week following treatment the younger plants produced about 40% less total alkaloids while the older plants displayed about a 32% increase in the total alkaloid content. The significantly increased alkaloid production in the older plants was due primarily to the favorable effect of G.A. in promoting more extensive growth. It was concluded that the age of the plant when treated appeared to be an important factor in causing increased growth which in turn resulted in increased alkaloid production per plant.

A favorable influence on growth and total alkaloid production was noted in *Datura stramonium* from a two-spray treatment at biweekly intervals of 100 p.p.m. of G.A. (143). The total dry weight was increased about 60% over controls and the stems indicated almost a twofold increase. The increases were not as great in stramonium receiving 1,000 p.p.m. of G.A. A single application with each concentration was less effective in promoting increased dry weights. In all cases root growth was less than in controls. The concentration of alkaloids in the leaves and tops and in the stems was significantly reduced by all of the treatments while an increase was found in the roots. Stramonium receiving a two-spray treatment of 100 p.p.m. of G.A. demonstrated the most favorable response, a 46% increase in total alkaloid production which was due primarily to increased growth. Brummett (145), in research which was awarded the 1959 Newcomb Award, found that a single 100-mcg. dose of G.A. induced responses in stramonium similar to that from a spray treatment. The height increases were not as great as with the latter treatment, but the growth rate as indicated from dry weight data obtained from weekly harvests was more rapid. Further, the roots demonstrated a beneficial effect (up to an 80% increase in root dry weight at the second harvest) contrasted with decreased growth from the spray treatments. Two factors may have contributed to increased root growth, the plants in this case were grown directly in the soil instead of pots, thus, "root-bounding" was not a possibility; and the plants received fertilizer. Significant decreases in the concentration of the alkaloids in the leaves (and tops) and in the stems were generally found at the first, second, and third weeks following treatment. The roots indicated slight changes. Slight decreases in the total alkaloid content per plant were induced in stramonium by the treatments. The roots of treated plants, however, demonstrated an increase of 54% at the third (final) harvest. This was due mainly to a significant increase in root growth.

In a later investigation, Brummett (146) studied the effects of G.A. on the growth, alkaloid concentration, and nitrogen fraction patterns in *Datura stramonium*. Two doses of G.A. were employed; *viz.*, 100 mcg. initially, and 25 mcg. at weekly intervals for 4 weeks. The plants in this study were at a later stage of development than in his previous work (145). Five consecutive harvests were gathered at weekly intervals. Gibberellin responses on growth were noted but the degree of response was less than when younger plants were employed. Maximal increases in leaves and stem weights at both dosage levels were observed at the end of the first week, whereas the roots required two weeks to attain a maximal response. The plants weighed slightly less than controls at the final harvest. However, the roots of the 25-mcg. series demonstrated increased weight throughout. The weekly dosage schedule elicited a more gradual response to the gibberellin effect than did an initial large dose.

The alkaloid concentration in the treated plant parts was reduced considerably at each harvest with both dosage levels. The total alkaloids per plant and plant part were increased considerably at the second harvest. This was due primarily to the increased weight of the plant. The reductions in total alkaloid content at the final harvest amounted to about 10 per cent.

Total plant nitrogen was decreased slightly, but this decrease was not considered significant until the last harvest. Significant decreases of approximately 50% in total plant nitrate nitrogen was observed, however. This decrease was most pronounced in the leaves. It was suggested that in the treated plants the nitrate nitrogen was not stored but was converted into ammonia

to be utilized by the plant in altered pathways of nitrogen metabolism. The changes in the total protein nitrogen of the leaves of the 100-mcg. group corresponded to changes in the total alkaloid content. The roots of this group displayed an inverse relationship during the first 2 weeks and a direct relationship for the last two harvests. No significant trend was noted in the stems. The aforementioned relationships were less conspicuous in plants receiving weekly dosages of G.A.

Fish conducted an intensive study in England on the effects of G.A. on growth and alkaloidal content of stramonium (147). The plants were grown under field conditions during the treatments and observation period. Five weekly treatments at three dosage levels, *viz.*, 20, 100, and 200 mcg., were employed using the injection technique. Plant material was harvested at 1, 2, and 5 weeks following the last treatment. A second experiment, conducted a year later, extended the 20- and 200-mcg. dosage levels over a longer growth period. This investigation differs from the previously reported ones (144–146) which were done under greenhouse conditions and plants were treated over a short growth period.

The following responses to the gibberellin treatment were reported (147): increase in height of treated plants was due to increased internode length, especially on the treated side of the plant; rapid growth in stem diameter caused axial splitting of the outer tissues resulting in the appearance of scar tissue; increases in dry weights of aerial parts, observed only in plants treated over a limited period, were due to greater stem development and did not vary with the dose of G.A. given; in plants treated at intervals over a long growing period, there was increased stem production but no gain in total dry weight; yields of leaves and tops in treated plants were not significantly different from those in control groups; significantly increased root weights were noted for a few groups of treated plants; the concentration of alkaloids was significantly reduced in the leaves and tops of the treated plants, the content being the lowest in the plants receiving the highest dose of G.A.

Ragan and Sciuchetti studied the effect of G.A. on the second generation of *Datura stramonium* (148). Seeds were collected in 1957 from stramonium plants which had received treatment of 1,000 p.p.m. of G.A. (A series) and 100 p.p.m. of G.A. (B series), and from untreated plants (controls). Plants were grown in pots containing soil in 1958 from these seeds under greenhouse condititions and were 57 days old when harvested. The "gibberellin effect" on growth was generally not observed. In some cases reverse effects were noted such as shorter plants than controls and decreased dry weights of the plant organs. The total dry weight of the A series was about 74% of the controls and that of the B series about 69%. The concentration of alkaloids in the various morphological parts of treated and untreated plants was about the same. The total alkaloid content (based on dry weights per plant and plant organ) was significantly less in series A and B. The decrease was due primarily to reduced growth. It was concluded that the "gibberellin effect" did not manifest itself in the second generation plants.

Table I summarizes the response of stramonium from a two-spray treatment with G.A. (143) and that of plants grown from seed obtained from that experiment (148). It should be pointed out that the favorable effect in the first generation from treatments was due to the more extensive shoot growth in spite of reduced concentrations in the shoots. On the other hand, the lower total alkaloidal content of the second generation plants was due to reduced growth and not to an effect on the concentration of alkaloids in the plant organs. Table II, likewise, summarizes the influence of G.A. on the growth and alkaloid production in the first-year (143) and second-year growth (144) of belladonna. In both cases the alkaloidal content was about half of the

Table I.—Influence of Gibberellic Acid on the First and Second Generation of *Datura stramonium*

Effect	First Year, %[a] (143)	Second Generation, %[a] (148)
Height	150	80
Growth	Taller, more mature	Shorter
Leaves	Longer, thinner, and chlorotic	Thinner and smaller
Dry weights		
Total	126	74
Leaves-tops	114	83
Stems	165	73
Roots	69	60
Alkaloid concentration	70 in shoots, 140 in roots	About the same in all plant parts
Total alkaloid content		
Per plant	108	83
Leaves—tops	99	76
Stems	132	69
Roots	96	67

[a] Per cent stated is that of control group; first generation plants received a two-spray treatment with 1,000 p.p.m. of G.A.

TABLE II.—COMPARISON OF EFFECTS OF GIBBERELLIC ACID ON THE FIRST AND SECOND-YEAR GROWTH OF BELLADONNA

Effect	First-Year, %[a] (143)	Second-year, %[b] (144)
Height	300 increase	20 decrease
Growth	Spindly and vinelike	Spindly
Leaves	Chlorotic and narrower	Narrower and thinner
Dry weights		
Total	101	50
Leaves—tops	78	56
Stems	260	50
Roots	72	43
Alkaloid concentration		
Leaves—tops	55	About the same
Stems	43	144
Roots	77	About the same
Total alkaloid content		
Per plant	53	55
Leaves—tops	40	55
Stems	113	72
Roots	51	43

[a] Per cent stated is that of control group; first year plants received 3 spray treatment with 1,000 p.p.m. of G.A.
[b] Average of two harvests.

controls. However, the reduced alkaloidal content in the first year was due primarily to significantly lower concentrations in the plant parts whereas that in the second-year growth was due mainly to reduced growth.

Stem tissue from *Datura stramonium* was cultured in a media to which has been added 10 p.p.m. of G.A. and inhibition of growth resulted (149). Very likely other investigators will employ the tissue culture techniques using G.A. on alkaloid-producing plants. The resulting research should be of considerable value.

Ambrose treated *Datura meteloides*[2] with four weekly doses of 25 mcg. of G.A. (150). Characteristic gibberellin effects were induced including increased height which amounted to 147 and 141% that of controls at the third and fourth week, respectively. The stems of treated plants appeared chlorotic while this was not so pronounced in the leaves. Analysis for chlorophyll a and b indicated a decrease in the leaves, tops and stems, at all harvest periods. This confirms the observations of other workers (143–147) who reported an apparent chlorotic effect. The growth was stimulated since increases in dry weights were generally noted throughout the observation period. At the final (fourth-week) harvest the total dry weight of treated plants was about 115% of controls and stem dry weight was about 140% of controls. The concentration of alkaloids in the plant parts was reduced considerably. In spite of a favorable effect on growth, the decreased concentration of alkaloids in the organs resulted in about a 10% reduction in total alkaloidal content per plant at the final harvest.

In an "Undergraduate Research Participation Program in Pharmacognosy" sponsored by a grant[3] from the National Science Foundation during the summer session of 1959 at Oregon State University, six participants conducted an extensive research project with *Datura stramonium*. The objectives of the research were twofold; first, to determine a concentration of G.A. which would produce the most favorable growth response; second, to determine whether frequency of application would elicit a different and/or more favorable response. The investigation employed 320 plants and was a $2 \times 4 \times 5$ factorial experiment in randomized blocks with eight replications. The first factor was the number of installments. In one case, the same dosage was applied once weekly; in the other, it was in two installments—half a dose given twice weekly. The second factor had four levels, mainly, 0, 12, 48, and 192 mcg. dosages of G.A. The third factor was the age of the plants at the time of harvesting and had five levels, namely, 0, 1, 2, 3, and 4 weeks. The following salient points were noted from a statistical analysis of data collected during the experiment: the greatest increase in height was noted in plants receiving the largest dosage of G.A. (192 mcg.). All treated plants grew more rapidly than controls. Height increases were significantly different at each dosage level (132). The most favorable response regarding height increase was somewhere between the 12-mcg. and 48-mcg. levels. Under the conditions of the experiment the frequency of application (whether one or two installments were used) did not significantly affect the height variable. The total dry weights of the treated plants were significantly affected by dosage and age. The number of installments had no significant effect. The growth rate varies with the dose of G.A. employed. Control plants grew at a slower rate than treated plants; this correlated with the trends shown by height data. It was the author's belief that if the number of installments had been extended over a longer period of time, e.g., 2 weeks instead of 1, the frequency of application may have been significant. This work will be published following a complete analysis for alkaloid and carbohydrate fractions.

[2] NSF Grant-G12131.

[3] NSF Grant-G8458.

Considerable interest has been evoked regarding the influence of G.A. on tobacco. Yabuta, *et al.* (151), in 1941 reported that gibberellin-treated tobacco seedlings demonstrated an immense increase in height and that the number of leaves was increased up to three times that of untreated. In a later publication (152) a decreased nicotine content in treated tobacco seedlings as well as a decrease in the average weight per plant was reported. Gray found that tobacco plants sprayed with G.A. produced leaves that were more elongated and pointed at the apex than those from untreated plants (89). Burk and Tso treated six species of *Nicotiana* with G.A. by six weekly injections of 15 mcg. of G.A. and found that at ten weeks the treated plants were about one-third taller than controls (153). The leaves on treated plants were longer, narrower, curled at the margins, and subject to sunscalding at the tips. In another set of experiments employing various concentrations of G.A. and several methods of treatment they found that treated tobacco plants contained considerably less alkaloids than controls. Further, they reported that the intensity of the effect increased with the amount of G.A. applied, and the alkaloid content decreased. However, no change in the composition of the alkaloids was observed. Parups found that treatment of tobacco with G.A. in field and greenhouse experiments had little effect on yield of leaves but decreased the nicotine content of both leaves and roots (154). It was postulated that the decrease of nicotine content in the tobacco leaves resulted from a change in the metabolism of roots caused by the G.A. application. Carew, in work conducted at the State University of Iowa with *Nicotiana rustica*, var. *Brasilia*, found that spraying ten-week-old plants with 25 and 100 p.p.m. of G.A. at 10-day intervals for 30 days and harvesting the plants two weeks after the final treatment resulted in a substantial increase in stem weight (155). Also a 10% increase in the total chlorophyll of leaves of treated plants was found. The concentration of nicotine in the leaves of treated plants was considerably less than controls.

A review of the literature on other species of plants producing alkaloids has indicated that very little has been done regarding the effect of G.A. in changing plant composition. For example, gibberellin treatment of larkspur (*Delphinium ajacis*) hastened flowering from 10 days to 2 weeks (130); opium poppy (*Papaver somniferum*) was studied for the effect of G.A. on flowering (156); the growth rate of *Coffea arabica* was increased three to fourfold when G.A. in concentrations of 50 to 100 p.p.m. was applied to the foliage of young seedlings (157); and, weekly applications of similar concentrations of G.A. to coffee leaves caused significant increases in growth (90). Coffee trees about 10 years old were sprayed with G.A. in concentrations varying from 1 to 100 p.p.m. (158). Within 10 days 60 to 70% of the buds opened when sprayed with concentrations above 20 p.p.m. and fruit set increased 400 to 700%. *Coffea arabica* seedlings treated monthly with G.A. demonstrated better growth than those given bimonthly applications (159). Differences in the number of leaves on the treated plants were not statistically significant. Plant height differences were significant at the 1% level. Yabuta, *et al.*, reported that tea (*Thea sinensis*) treated with gibberellin produced longer tea buds than untreated plants and that the total yield of tea leaves was not changed (160). Analyses for tannin, caffeine, ascorbic acid, total N, crude fiber, and soluble matter indicated slight differences from those of the control.

Considerable value would accrue from extensive investigations on a variety of plants which produce alkaloids. The use of G.A. by the pharmacognosist and allied workers could divulge important fundamental information regarding the biogenesis of alkaloids. Also some of the findings may disclose practical applications in drug plant propagation.

METABOLIC EFFECTS IN OTHER PLANTS

The effect of G.A. on plant composition and plant metabolism have been adequately covered through 1958 in other reviews (1–3). Some of the developments since that time and pertinent information will be presented here.

In many cases plants treated with G.A. increase their height, leaf area, and dry weight. The increased dry weight may be due to an increase in photosynthetic activity or more efficient utilization of photosynthetic products. No clear-cut linkage with any metabolic pathway has yet been established, but analyses so far have always shown the greatest changes among carbohydrate constituents (2). Conflicting reports appear in the literature regarding the effect of G.A. on photosynthesis, respiration, and transpiration. Coulombe has indicated a rapid increase in respiration, photosynthesis, and transpiration of tomato foliage following a spay-treatment (161). A maximum rate was reached about 6 hours after spraying but was followed by a rapid decline. Except for transpiration, which returned to its initial rate, the respiration

and photosynthesis of the treated plants remained higher than controls. Alvim reported that net assimilation rate and relative growth rate of beans were both increased by G.A., and implied that the amount of dry matter produced by photosynthesis per unit of leaf area was significantly higher in plants receiving G.A. (162). Increased respiration of seeds by G.A. has been reported (163, 164). Hayashi in his study on the effects of G.A. treatment on whole plants and attached leaves reported that photosynthetic activity of tomato and rice plants was increased but no difference was noted on the photosynthetic activity of attached tomato leaves (165). The total and reducing sugar content of the shoots of rice plants was increased while the total sugars of the roots were decreased.

On the other hand, reduced photosynthetic activity or no apparent effect on photosynthesis from G.A. have been reported. Haber and Tolbert working on detached leaves noted that G.A. neither enhanced the rate of carbon dioxide fixation per unit of leaf tissue nor altered the general pathways of short time metabolism of the newly fixed $C^{14}O_2$ in the sugars, organic acids, and amino acid products (166). Haber, *et al.*, in a later experiment reported that in light-grown seedlings which are prevented (by darkness) from performing photosynthesis, G.A. increased their dry weights over water-control seedlings (167). They claimed that the effect of G.A. in their experiment could not be attributed to either direct or indirect actions on photosynthetic fixation. It was also noted that the increases in total carbon, soluble carbohydrate, total nitrogen, and protein in G.A.-treated shoots were approximately proportional to the dry weight increase. Fang, *et al.*, employing C^{14}-labeled compounds found that G.A. increased slightly $C^{14}O_2$ production from administered IAA-1-C^{14} in corn root tissues (168). The formation of indoleacetylaspartate was greatly inhibited and consequently the free IAA level in G.A.-treated tissues was increased by 15% over the control tissues. The catabolic pathways of glucose and acetate were not found to be affected to any extent by the short-term G.A. treatment. However, the treatment slightly increased the catabolic oxidation of C-1 and C-2 carbons of acetate (168). In spite of conflicting reports about the effects of G.A. on photosynthesis and respiration, considerable knowledge has been gained thus far. Future research should clarify this aspect and provide us with a better understanding of the role that G.A. plays in these important plant processes.

Several reports have been made concerning the effects of G.A. on enzymatic activity of plants. Kato and Katsumi (169) and Brian and Hemming (170) have reported that gibberellin treatment of either pea seedlings or pea internode sections did not affect the IAA oxidase activity in plant tissue. On the other hand, Pilet has shown that the IAA oxidase activity of carrot tissues (171) is reduced by G.A. as well as in *T. ocholeucum* (172). Watanabe and Stutz found that G.A. treatment of the terminal buds of lupine plants over a period of 1 month resulted in a decrease in IAA oxidase activity in bud and stem tissues (173). Their results are in agreement with the three-factor system in which G.A. has an auxin-sparing action by regulating the concentration of a natural growth inhibitor.

G.A. appears to stimulate enzymatic activity in other cases. The work performed on barley seeds is of interest. Several have reported that gibberellin treatment hastened the germination of barley (174–177). Paleg has reported that G.A. increased the activity of beta-amylase and the beginning of alpha-amylase activity as well as increasing the liberation of reducing sugars (178, 179). In addition to activating growth in barley during the early stages of malt processing, gibberellin caused an increase in the color of malt and the various enzyme systems of barley (180). G.A. induced a large increase in the glutamico-oxalacetic-transaminase and glutamic-pyruvate-transaminase content of germinating barley seeds (181). The activity of various phenol oxidases was increased following gibberellin treatment of tomato seeds (182). Application of G.A. to young beans increased the phosphatase activity in the leaves and stems and for beta-amylase in the roots; decreased the activity for beta-amylase in the leaves and stems and for pectin methylesterase in the roots (183).

Roots of bean seedlings immersed in a solution of $H_3P^{32}O_4$ and treated with G.A. absorbed more phosphorus than untreated plants (184). On the other hand, young corn seedlings fed radioactive phosphorus as $Ca(H_2P^{32}O_4)_2$ and treated with gibberellin demonstrated decreased phosphate uptake in the shoots while the sugar in the leaves was increased 2.8-fold (185). Seeds from the gibberellin treated plants demonstrated a higher sugar content and increased weight.

Recent reports on plant composition include the following: Ergle found variable effects in young cotton plants on the concentration of sugars, starch, hemicellulose, and various nitrogen fractions depending upon the concentration of G.A. employed and the plant part analyzed (186).

Dure and Jensen have also reported on the response of cotton embryos to G.A. treatment (187). Protein content of alfalfa (188), and of sorghum (189) were increased by G.A. Finn and Nielson found that gibberellin decreased the protein content of all grasses and legumes treated but, due to increased yields, total nitrogen in the dry matter was increased (190). Morgan and Mees, likewise, found that the nitrogen content of grass was slightly reduced (191). Mes found that increased dry weight and total nitrogen content resulted from spraying three-week-old *Vicia villosa* with G.A. (192). Wittwer and Bukovac found that G.A. accelerated the growth of Kentucky bluegrass; did not significantly alter the percentage of dry matter or mineral composition; and significantly reduced the total sugars (193). Although diverse responses have been induced in total plant nitrogen, the general trend appears to be a decrease in total nitrogen from G.A.

In most cases the influence of gibberellic acid appears to be greater on carbohydrate metabolism (2, 3, 127, 131, 133, 137). Recent work with sugar cane indicates a reduced sucrose content per plant from G.A. treatment but an increase in tons per acre (due to increased growth) without changing the yield per acre (107). On the other hand, the growth rate of mother shoots of sugar cane was increased by G.A. as well as the sucrose content but a decrease in nitrogen content was noted (194). Stout found that G.A. applied to the growing points of field-grown sugar beets reduced the sugar percentage and respiration rate of root tissue (195). Also foliar sprays of G.A. to the leaves had no apparent effect on the dry weight, total nitrogen, or respiration rate of disks cut from treated leaves within one week of treatment (195). The review by Wittwer and Bukovac (3) lists six references to indicate that the yields of sugar beets and sugar cane have not been significantly increased without corresponding reducation in per cent sucrose.

A chlorotic effect of the leaves is usually noted in many plants following treatment with G.A. This might indicate that G.A. influences chlorophyll synthesis or that the apparent paleness of leaves is due to dilution. Wolf and Haber found that wheat seedlings grown in a G.A. solution contained about the same content of total chlorophyll as controls 6 days after treatment, but, about 20% less at 12 days (196). The carotenoid reductions were about the same as that for chlorophyll. The ratio of chlorophyll a to chlorophyll b remained about the same in treated and untreated seedlings. Thirty-day-old seedlings grown about 3 weeks in soil demonstrated about a 70% increase in height, a 23% reduction in fresh weight, and a 36% decrease in total chlorophyll. The authors concluded that in young plants G.A. exerted a pronounced effect on growth without affecting the total content, anabolism, or catabolism of chlorophyll, and that the pale appearance of the six-day-old treated plants was due solely to pigment dilution (196). Sestak and Ullman report that eight-day-old etiolated seedlings of wheat and corn grown in a nutrient solution with G.A. produced a lower content of chlorophyll a and b and carotenoids after 24 hours of illumination (197). Among the medicinal plants, the per cent of chlorophyll in treated plants has been shown to be less in spearmint (125) and *Datura meteloides* (150) and greater in peppermint (127), foxglove (131), buckwheat (137), and tobacco (155). In both plants demonstrating a decrease in chlorophyll per-cent, considerable increases in dry weights were noted. The latter trend was generally reported also for medicinal plants, demonstrating an increase in the per cent of chlorophyll. It would appear to the reviewer that the apparent paleness of leaves from gibberellin treated plants is most likely due to dilution rather than a direct effect on chlorophyll synthesis.

SUMMARY AND CONCLUSIONS

Favorable growth responses have been induced in some medicinal plants by G.A. In most cases increased dry weights have been reported. However, a reduction in the per cent of active constituents in plants producing volatile oils and alkaloids is the usual trend. On the other hand, increases in the carbohydrate content have usually resulted in an increase of the per cent of glycosides and the total glycoside content. If a favorable response on growth could be induced in alkaloid-producing plants without a corresponding reduction in the concentration of alkaloids, G.A. would have practical applications in increasing the yield from medicinal plants. It is the reviewer's opinion that the discouraging results obtained thus far in the few investigations conducted with the mints should not be taken as conclusive evidence that favorable responses cannot be induced under the right conditions. As previously mentioned, a number of factors such as environmental conditions, nutrient requirements, the age of the plant when G.A. is applied, dosage and/or frequency of application, method of application, and generic differences must be studied in more detail before final conclusions can be made whether G.A. has practical applications in pharmacognosy. Limited experiments

on the germination of seeds of medicinal plants have been most encouraging. The germination rate and total germination as well as a more uniform emergence of seedlings has been induced by G.A. This area of research could lead to fruitful results especially when seeds are difficult to obtain and/or seed germination offers an obstacle to the satisfactory propagation of the species.

Of more importance are the contributions of a basic nature which might accrue. Fundamental information on the natural plant processes such as germination, growth, and flowering have been divulged during the past few years from the intensive studies conducted with G.A. It is hoped, likewise, that a better understanding about the biogenesis of the active components of medicinal plants might result from research in this fascinating area. Possibly we may have an answer as to **why** and **how** belladonna produces atropine, or digitalis its array of glycosides.

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Determination of Lipase Activity in Pharmaceutical Preparations Intended for Oral Administration

By EDGAR A. LAZO-WASEM

A practical, reliable procedure for the standardization of lipase activity in pharmaceutical preparations is described. By this method, which reproduces as closely as possible the conditions under which fat is digested in the body, one can test the effectiveness of the enteric coating to protect against gastric destruction of lipase.

MANY PROCEDURES for the standardization of lipase activity in pharmaceutical preparations are based on methods such as that of Willstatter, *et al.* (1), in which titratable acidity is measured following a standardized digestion period of an edible oil. The main disadvantage of the classical Willstatter procedure is the fact that measurement of fatty acids released during digestion is made by titrating the oil-in-water emulsion with an aqueous alkaline hydroxide solution. Titrations of oil-in-water emulsions are difficult to reproduce and carry with them errors introduced by the subjective evaluation of the end point.

More reproducible determinations of liberated acidity can be obtained by extracting the fatty acids from the digestion mixture with a solvent and then titrating with an alcoholic solution of sodium hydroxide. Titrations of benzene-extracted fatty acids carried out with alcoholic sodium hydroxide yield sharp, reproducible, easily detectable end points when using alcoholic indicator solutions.

The purpose of this report is to describe a method which has been found entirely reliable and reproducible over a period of several years. In our method, which follows the suggestions of Sammons, Frazer, and Thompson (2), conditions *in vitro* were made to resemble as closely as possible those which occur *in vivo* during normal fat digestion. The substrate selected is one of the common dietary long-chain triglycerides, olive oil, rather than a preformed emulsion or short-chain glyceride. The mixing properties of intestinal motility were reproduced by agitation, bile constituents were added at a concentration normally found in the duodenum, and incubations were carried out at the temperature of the human body, 37°.

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EXPERIMENTAL

Special Apparatus.—A constant temperature bath equipped with a mechanical shaking device (Labline model 3580) is used. The shaking rate of the bath is regulated to approximate 200 vibrations per minute.

Reagents.—Sodium hydroxide, 0.05 *N*, in methyl alcohol. Olive oil U. S. P. (Pompeian Olive Oil Corp., Baltimore, Md.). Phenolphthalein indicator in alcoholic solution. Benzene (reagent grade, thiophene free). pH 7.8 Phosphate buffer: dissolve 0.54 Gm. of anhydrous monobasic potassium phosphate in sufficient water to make 50 ml.; dissolve 4.73 Gm. of anhydrous dibasic sodium phosphate in sufficient water to make 500 ml.; mix 10 ml. of the first solution with 90 ml. of the second solution and adjust the pH, if necessary, by adding more of either solution. Bile salt solution: dissolve 0.500 Gm. of extract of ox bile N. F. powder in sufficient pH 7.8 phosphate buffer to make 100 ml. of solution.

Preparation of Test Solutions.—The samples to be tested are diluted with cold distilled water and homogenized in an electric blender (Waring Blendor model 702A) for about one minute. Final test solutions are made to contain 1 unit/ml. These solutions are stable for at least two hours when stored in a refrigerator.

Procedure.—Samples measuring 0.4, 0.6, and 0.9 ml. of the solution to be tested are pipetted into flasks of approximately 100-ml. capacity and the volume in each flask adjusted to 1 ml. with distilled water. A reagent blank is also set up using 1 ml. of distilled water. Bile salt solution, 5 ml., is then added to each flask and, in turn, 1 ml. of olive oil is delivered by means of a 1-ml. syringe. The mixture is then incubated, with shaking, at 37° for thirty minutes.

At the end of the incubation period, 5 drops of concentrated hydrochloric acid are added to each flask to stop the enzymatic action, followed by 20 ml. of benzene. The flasks are then shaken by hand for one minute and allowed to stand until the two phases separate. A 10-ml. aliquot of the upper benzene layer is pipetted into a 50-ml. flask. A 1-ml. quantity of methyl alcohol and 3 drops of alcoholic phenolphthalein indicator are added and the contents of the flask titrated with 0.05 *N* sodium hydroxide in methyl alcohol. The titration volume in milliliters is recorded for each level of unknown test solution and for the blank.

Definition of Unit.—One Wilson unit of lipase activity will liberate an amount of fatty acid equivalent to 1 ml. of 0.05 *N* alcoholic sodium hydroxide solution from 1 ml. of olive oil in thirty minutes under the conditions of the assay.

Calculation of Lipase Activity.—Lipase activity (units/Gm.) = 2 (unknown titration − blank titration)/Gm. of sample per digestion flask.

The three values obtained should agree within 5% of the average to indicate linearity of response. If not within such limits the test dilutions are changed and the assay is repeated. The average value of the three levels is used to assign potency.

Using the described method, the lipase activity of various commercially available lipase preparations was determined.

Since it has been shown that lipase is destroyed by gastric juice (3), lipase activity in enteric coated and nonenteric coated dosage forms after incubation in digestive fluids was also determined. For this experiment three dosage forms, all commercially available, were selected. Product A was enteric coated forms labeled to contain 300 mg. pancreatin N. F. per tablet, corresponding to 150 lipase units on the basis of the data in Table I. Product B was also enteric coated forms labeled to contain 150 lipase units per tablet. Product C was capsules labeled to contain 300 mg. of product corresponding to 2,000 manufacturer's own lipase units per capsule. On the basis of our own assays, each capsule was established to contain 690 lipase units by the present method.

All dosage forms were allowed to incubate in 5 ml. simulated gastric fluid U. S. P. at 37°. Ten minutes later, 2 volumes of U. S. P. simulated intestinal fluid (without the pancreatin) were added and incubation continued for another ten minutes. Lipase assays were performed on aliquots of the gastric-intestinal fluid mixture. Incubations were also carried out in simulated intestinal fluid U. S. P. (less pancreatin) only, without first exposing the dosage form to the gastric fluid.

RESULTS AND DISCUSSION

In the course of experimenting with this method we confirmed the finding of Sammons, *et al.* (2), to the effect that, within certain limits, shaker speed bears a definite relationship to the amount of fatty acids liberated. We standardized at a speed of 200 vibrations per minute because with our shaker bath, such rate of vibration gave optimal results. Since the mixing of the digestion mixture is affected not only by rate of vibration but also by length of the stroke of the shaking mechanism, it is recommended that each individual laboratory establish optimal vibration rate for their particular equipment.

Table I shows the comparative lipase activity of various commercial preparations by the described method.

The results in Table II unequivocally show the necessity of an enteric coat in protecting lipase when exposed to gastric fluid. The protection of lipase by enteric coating has been previously demonstrated (4).

Table I.—Comparative Lipase Activity of Various Commercial Products

Product	(Wilson) Lot	Potency (Units/Gm.)
Pancreatin N. F.	108309	460
Pancreatin N. F.	108737	540
Pancreatin 4 × N. F.	108441	2,060
Pancreatin 4 × N. F.	113349	2,400
Lipase 2,000	109751	2,190
Lipase 4,000	109302	4,740
Lipase 16,000	112355	16,800

Table II.—Lipase Activity of Three Dosage Forms after Incubation in Simulated Gastric Fluid Plus Simulated Intestinal Fluid Compared to Incubation in Simulated Intestinal Fluid Alone

Dosage Form	Commercial Product	Lipase Potency (Units/Tablet or Capsule) Gastric Fluid plus Intestinal Fluid	Intestinal Fluid Alone
Enteric coated tablets	A	150	150
Enteric coated tablets	B	150	150
Capsules (not enteric coated)	C	30	690

The potency of different lipase preparations in terms of their power to liberate fatty acids from an olive oil-digestion mixture cannot be compared with reservations between any two methods unless one has predetermined that the two methods give equivalent results. The temperature and pH of the incubation fluid, the length of the digesting period, and the type and amount of the enzyme activator used have most significant effects on lipase activity. In the present method ox bile, which contains cholic and desoxycholic conjugates of taurine and glycine, is used as the lipase activator in an attempt to imitate physiological conditions. It should be pointed out, however, that when activating lipase by addition of certain bile salts as sodium desoxycholate (5) and miscellaneous lipase stabilizers, such as calcium ions, in amounts far in excess of those believed present physiologically, increments in fat digestions as high as two times those obtained by the method reported here can be brought about.

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Steroidal Sapogenins LX

Survey of Plants for Steroidal Sapogenins and Other Constituents

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This is a report of the chemical examination of the sixth 1,000 accessions in a survey of plants for steroidal sapogenins. Data are given for about 1,030 species in 128 families, practically all collected in the United States. In *Agave* a new record of 4.4% genin, mostly smilagenin, was established. The collections of *Dioscorea composita* were uniformly high in diosgenin, with a new record of 10.1%. *D. spiculiflora* regularly contained diosgenin and yamogenin, and the highly desirable gentrogenin and correllogenin. A sample of seed of a *Yucca* sp. gave 11.1% sarsasapogenin. The recently discovered willagenin was found again in a *Yucca* sp. Of the total species examined, about 10% contained alkaloids, some 64 of them new to the record. This is the final report on this project of plant screening.

THIS is a continuation of the previous reports (1–8) on the first five 1,000 plant accessions in our survey of plants for steroidal sapogenins and other constituents. It is the final report on this project and covers about 1,030 species in 128 families. A few accessions (with accession numbers less than 5,000) from previous lists are included because repeat analyses showed the presence of alkaloids.

PROCUREMENT

With the publication of these results, the general sample procurement and chemical screening phase of the U. S. Department of Agriculture's search for plant sources of steroidal sapogenins useful in the elaboration of cortisone comes to a close. In the 12 years that have elapsed since the beginning of the program when Dr. John T. Baldwin explored West Africa for species of *Strophanthus* and *Dioscorea*, over 6,000 plant samples, representing 208 families and 1,397 genera, have been collected from nearly all parts of the world for chemical analysis at the Eastern Utilization Research and Development Division. Over two-thirds of the families of *Angiospermae* recognized in Engler and Prantl's "Die natürlichen Pflanzenfamilien" are represented. Obviously some of the families are very inadequately represented and it cannot be said that any has been thoroughly sampled. For the purposes of a screening program, however, coverage has been sufficiently broad and, in the more promising families, sufficiently deep to indicate that the chances are slim indeed of finding better sources of steroidal sapogenins than have been found in the genus *Dioscorea*. Species of *Agave* and *Yucca*, though some are as rich in steroidal sapogenins as *Dioscorea*, do not compare favorably with the latter in crop potential. Efforts are now being concentrated on fashioning a successful crop from species of *Dioscorea*.

Samples reported in Table I were collected, with few exceptions, from four areas: Mexico, Central America, southwestern United States, and the West Indies. This is not entirely fortuitous for these areas are rich in members of the three families represented. Table II, reporting on 594 genera in 128 families, reveals that the heaviest concentration of collecting activity was in the Atlantic Coast states with the Southwest and Pacific Coast states more heavily represented than in previous reports of this series.

Plant identification work, which could not keep pace with the rapid expansion of the program in earlier years, has now provided names for most of the species studied. Some taxonomically difficult genera such as *Agave* and *Dioscorea* are subjects of monographic study by botanists of the New Crops Research Branch. As these studies progress, authoritative specific determinations will be made for those now listed by generic name only.

RESULTS

Table I lists the plants containing steroidal sapogenins, mostly *Agave*, *Dioscorea*, and *Yucca*, but with one *Ruscus*. Tests for nonsteroidal constituents were not made on most of these and, where made, nothing noteworthy was found.

Table II lists the plants not containing steroidal sapogenins and shows the results of tests for saponin, flavonoids, alkaloids, and tannins.

As to steroidal sapogenins, there are several items of note. The 4.4% in 6362, *Agave* sp., is the highest in our experience, the previous highs being 3.3% to 3.6%. The 3.6% in 6382, *Agave lecheguilla*, is noteworthy in that the material was the leaf pulp after scutching out the fiber.

Dioscorea composita has always been a good, but erratic, source of diosgenin, having varied from zero to a previous record of 10.1%. The present collections of this species are uniformly high and include a new record of 13.2%. *D. spiculiflora* is the one species which contains the two recently discovered genins, gentrogenin and correllogenin (9), with highly desired chemical properties for the production of cortisone. These genins seem always to be accompanied by diosgenin and yamogenin. In most

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TABLE I.—PLANTS CONTAINING STEROIDAL SAPOGENINS

Code for Sapogenins (the Suffix "Genin" Omitted)

co—corrello
d—dios
dm—9-dehydromano
g—gito
ge—gentro
h—heco
k—kammo
m—mano
nr—neorusco
r—rusco
sm—smila
ss—sarsasapo
t—tigo
w—willa
y—yucca
ya—yamo
diOH—unidentified dihydroxy genin
C=O—unidentified carbonyl genin

Code for Plant Parts

b—bark
fd—frond
fl—inflorescence
fr—fruit
l—leaf
r—root
s—stem
sd—seed
t—tuber
tw—twig
w—whole plant above ground
wd—wood
y—young

Code for Source Names

C. G. F.—U. S. Plant Introduction Garden, Coconut Grove, Fla. (cult.)
Chico, Calif.—U. S. Plant Introduction Garden, Chico, Calif. (cult.)
G. D. M.—U. S. Plant Introduction Garden, Glenn Dale, Md. (cult.)

Other Codes

ni—not identified
tr—trace

Accession No.	Species	Collection: Source	Collection: Date Collected	Collection: Plant Part	Hemolysis	Sapogenins: Total, M.F.B. %	Sapogenins: Identified, % of Total
		AMARYLLIDACEAE					
6024-O	*Agave ferdinandi-regis*	Kingston, Jamaica	10/57	l		...	...
6379	*Agave lecheguilla*	Belle Glade, Fla.	7/57	l, dead	+	1.7	sm100
6380	*Agave lecheguilla*	Belle Glade, Fla.	7/57	l	+	1.2	sm100
6381	*Agave lecheguilla*	Belle Glade, Fla.	7/57	l bud	+	1.6	sm100
6382	*Agave lecheguilla*	Belle Glade, Fla.	7/57	l, bagasse	+	3.6	sm100
6383	*Agave lecheguilla*	Belle Glade, Fla.	7/57	l, bagasse	+	3.4	sm100
6017-O	*Agave morrisii*	Kingston, Jamaica, B. W. I.	10/57	l	−	...	...
6314	*Agave vilmoriniana*	Sonora, Mexico	5/57	l, dead	+	0	...
6360	*Agave sp.*	Sonora, Mexico	6/57	l, dead	+	1.0	sm95; y5
6361	*Agave sp.*	Sonora, Mexico	6/57	l, dead	+	0.7	sm92; y8
6362	*Agave sp.*	Sonora, Mexico	6/57	l, dead	+	4.4	sm84; y16
6363	*Agave sp.*	Sonora, Mexico	6/57	l	+	1.4	sm63; y37
6364	*Agave sp.*	Sonora, Mexico	6/57	l	+	0.4	sm
6365	*Agave sp.*	Sonora, Mexico	6/57	l, dead	+	1.0	g44
6366A	*Agave sp.*	Sonora, Mexico	6/57	fl	+	1.4	m + dm37
6366B	*Agave sp.*	Sonora, Mexico	6/57	fl, s	+	0	...
6387	*Furcraea cabuya*	Belle Glade, Fla.	7/57	l, dead	−	...	...
6391	*Furcraea gigantea*	Belle Glade, Fla.	7/57	l, dead	−	...	...
6018-O	*Furcraea selloa*	Kingston, Jamaica, B. W. I.	10/57	l	−	...	...
6393	*Furcraea sp.*	Belle Glade, Fla.	7/57	l, dead	−	...	...
		DIOSCOREACEAE					
5264	*Dioscorea alata*	Leon Co., Fla.	10/54	l, s, t	−	...	...
5844	*Dioscorea alata*	Santiago de Las Vegas, Cuba	2/56	t	+	0	...
5245	*Dioscorea batatas*	Frederick Co., Md.	10/54	l, s, t	−	...	...
6132	*Dioscorea bulbifera*	Savannah, Ga.	10/56	t	+	0	...
5334	*Dioscorea cayenensis*	Mayaguez, Puerto Rico	4/55	t	+	0.2	ni
5778	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	0.8	d100
5779	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	2.0	d100

5780	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	6.4	d100
5781	*Dioscorea composita*	Oaxaca, Mexico	12/55	t	+	5.4	d100
5782	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	4.0	d100
5783	*Dioscorea composita*	Oaxaca, Mexico	12/55	t	+	6.2	d100
5784	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	4.7	d100
5785	*Dioscorea composita*	Oaxaca, Mexico	12/55	t	+	6.0	d60; ya40
5786	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	5.4	d100
5787	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	3.5	d90; ya10
5788	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	4.4	d100
5789	*Dioscorea composita*	Veracruz, Mexico	12/55	t	+	6.5	d100
6257	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	5.1	d95; ya
6258	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	6.8	d95; ya
6259	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	1.7	ni
6260	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	4.3	d, ya
6261	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	7.0	ni
6262	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	5.2	d100
6263	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	5.6	d, ya
6264	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	3.5	d100
6265	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	5.3	d100
6266	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	5.8	d100
6267	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	3.4	d, ya
6268	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	6.8	d, ya
6269	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	13.2	d89
6270	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	5.3	d, ya
6271	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	5.6	d, ya
6272	*Dioscorea composita*	Veracruz, Mexico	4/57	t	+	4.7	d88; ya
5595	*Dioscorea convolvulacea*	Turrialba, Costa Rica	8/55	t	−	...	...
5774	*Dioscorea floribunda*	Veracruz, Mexico	12/55	t	+	3.6	d50; ya50
5775	*Dioscorea floribunda*	Veracruz, Mexico	12/55	t	+	4.0	d + ya100
5776	*Dioscorea floribunda*	Veracruz, Mexico	12/55	t	+	2.6	d50; ya50
5777	*Dioscorea floribunda*	Veracruz, Mexico	12/55	t	+	6.1	d60; ya40
6051	*Dioscorea floribunda*	G. D. M.	8/56	t	+	1.4	ni
6225	*Dioscorea floribunda*	Guatemala	3/57	t	+	5.2	d75; ya
6226	*Dioscorea floribunda*	Guatemala	3/57	t	+	1.6	d93; ya
5315	*Dioscorea Mexicana*	G. D. M.	3/55	t	+	0.03	d55; ya
5845	*Dioscorea Mexicana*	Costa Rica	3/56	t	+	0.16	ni
5317	*Dioscorea spiculiflora*	Chiapas, Mexico	3/55	t	+	3.6	d + ya65; ge + co35
5767	*Dioscorea spiculiflora*	Chiapas, Mexico	12/55	t	+	3.7	d + ya70; ge + co30
5768	*Dioscorea spiculiflora*	Chiapas, Mexico	12/55	t	+	5.5	d + ya50; ge + co50
5769	*Dioscorea spiculiflora*	Chiapas, Mexico	12/55	t	+	4.7	d + ya45; ge + co55
5770	*Dioscorea spiculiflora*	Chiapas, Mexico	12/55	t	+	3.8	d + ya60; ge + co40
5771	*Dioscorea spiculiflora*	Chiapas, Mexico	12/55	t	+	3.0	d + ya65; ge + co35
5772	*Dioscorea spiculiflora*	Chiapas, Mexico	12/55	t	+	2.0	g + ya55; ge + co45
5773	*Dioscorea spiculiflora*	Chiapas, Mexico	12/55	t	+	5.0	d + ya45; ge + co55
6273	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	5.0	ge + co44; d, ya
6274	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	5.9	ya + co12; d, ya
6275	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	4.2	ge + co80
6276	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	6.0	d + ya70; ge, co
6277	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	2.0	ge + co33; d, ya
6278	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	3.1	ge + co45; d, ya

Accession No.	Collection: Species	Source	Date Collected	Plant Part	Hemolysis	Sapogenins: Total, M.F.B. %	Identified, % of Total
6279	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	3.2	ge + co23; d, ya
6280	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	0.8	ni
6281	*Dioscorea spiculiflora*	Chiapas, Mexico	4/57	t	+	3.9	ge + co55; d, ya
6425	*Dioscorea villosa*	Marshall, Ill.	8/57	t	+	1.3	ni
5172	*Dioscorea sp.*	G. D. M.	9/54	r	−	...	...
5308	*Dioscorea sp.*	Santa Cruz, Bolivia	1/55	t	−	...	...
5314	*Dioscorea sp.*	G. D. M.	3/55	t	−	...	...
5316	*Dioscorea sp.*	G. D. M.	3/55	t	−	...	...
5344	*Dioscorea sp.*	Tallahassee, Fla.	4/55	w	+	1.0	d100
5790	*Dioscorea sp.*	Veracruz, Mexico	12/55	t	+	0	...
4791	*Dioscorea sp.*	Veracruz, Mexico	12/55	t	+	0	...
5792	*Dioscorea sp.*	Chiapas, Mexico	12/55	t	+	2.9	d65; ya35
5815	*Dioscorea sp.*	G. D. M.	1/56	t	+	0	...
5816	*Dioscorea sp.*	Puerto Rico	1/56	t	+	6.1	d100
5817	*Dioscorea sp.*	Puerto Rico	1/56	t	+	2.8	ni
5924-O	*Dioscorea sp.*	British Honduras	1/57	t	+	0	...
6025	*Dioscorea sp.*	Turrialba, Costa Rica	7/56	t	+	3.4	ni
6026	*Dioscorea sp.*	Turrialba, Costa Rica	7/56	t	+	3.29	ni
6221	*Dioscorea sp.*	British Honduras	3/57	t	+	0	...
6222A	*Dioscorea sp.*	British Honduras	3/57	t	+	0	...
6222B	*Dioscorea sp.*	British Honduras	3/57	s	+	0	...
6223	*Dioscorea sp.*	San Sebastian, Guatemala	3/57	t	+	0	...
6224	*Dioscorea sp.*	Guatemala	3/57	t	+	4.7	d81; ya
6227	*Dioscorea sp.*	Guatemala	3/57	t	+	6.2	d75; ya
6228	*Dioscorea sp.*	Costa Rica	3/57	t	+	4.0	d70; ya
6229	*Dioscorea sp.*	Turrialba, Costa Rica	3/57	t	+	1.5	d93; ya
6230	*Dioscorea sp.*	Turrialba, Costa Rica	3/57	t	+	1.7	d86; ya
6294	*Dioscorea sp.*	British Honduras	5/57	t	+	0	...
6295	*Dioscorea sp.*	British Honduras	5/57	t	−	...	...
6296	*Dioscorea sp.*	British Honduras	5/57	t	−	...	...
6297	*Dioscorea sp.*	British Honduras	5/57	t	−	...	...
6402	*Dioscorea sp.*	Brazil	7/57	r	−	...	...
6403	*Dioscorea sp.*	Brazil	7/57	r	−	...	...
6404	*Dioscorea sp.*	Brazil	7/57	r	−	...	...
		LILIACEAE					
6401	*Ruscus aculeatus*	Bologna, Italy	7/57	r	+	0.12	r, nr
5813A	*Yucca Australis* (*Y. filifera*)	Huntington, Calif.	12/55	l	+	...	...
5813B	*Yucca Australis* (*Y. filifera*)	Huntington, Calif.	12/55	r, crowns	+	0.19	ss100
6317	*Yucca baccata* var. *brevifolia* (*Y. Arizonica*)	Sonora, Mexico	5/57	fr	−	...	...
5934A	*Yucca baccata* var. *macrocarpa* (*Y. torreyi*)	Brewster Co., Tex.	5/56	empty pod	+	1.2	ss100
5934B	*Yucca baccata* var. macrocarpa (*Y. torreyi*)	Brewster Co., Tex.	5/56	sd	+	1.2	ss100
6283	*Yucca baccata* var. *macrocarpa* (*Y. torreyi*)	Brewster Co., Tex.	4/57	fl	+	1.9	ss100
6284	*Yucca baccata* var. *macrocarpa* (*Y. torreyi*)	Brewster Co., Tex.	4/57	s	+	1.3	ss100
5935-O	*Yucca brevifolia*	W. Caliente, Nev.	5/57	s, fl	+	1.7	t + h32
5923	*Yucca elata*	Brunswick Co., Tex.	5/56	y, fl	−	...	...
5227-O	*Yucca smalliana* (*Y. filamentosa*)	Montgomery Co., Pa.	10/54	sd	+	0.5	g70; t30

6367	*Yucca sp.*	Bacanora, Mexico	6/57	wd	+	0.3	ss
6368	*Yucca sp.*	Bacanora, Mexico	6/57	l	+	1.4	C=O 57; ss38; diOH
6369	*Yucca sp.*	Bacanora, Mexico	6/57	l, dead	..	0.4	ss55; w30
6370	*Yucca sp.*	Bacanora, Mexico	6/57	l, dead	+	0.2	h40; g, k
6371A	*Yucca sp.*	Bacanora, Mexico	6/57	sd	..	11.1	ss99
6371B	*Yucca sp.*	Bacanora, Mexico	6/57	pod	..	1.8	ss98
6372	*Yucca sp.*	Sonora, Mexico	6/57	l	+	0.9	ss98; diOH

TABLE II.—PLANTS NOT CONTAINING STEROIDAL SAPOGENINS

(For codes see Table I)

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		ACANTHACEAE						
5876	*Aphelandra deppeana*	Atenas, Costa Rica	4/56	l, s, b	−	0	0	..
5907	*Aphelandra sinclairiana*	Los Diamantes, Costa Rica	5/56	l, s	−	0	0	..
5912	*Aphelandra tetragona*	Palmar, Costa Rica	5/56	l, s	−	0	0	..
5621	*Carlowrightia linearifolia*	Jeff Davis Co., Tex.	8/55	l, s	−	0	0	..
5715	*Dianthera sp.*	Val Verde Co., Tex.	9/55	l, s	−	0	0	..
5411	*Dicliptera halei*	Jefferson Co., Fla.	7/55	w	−	0	0	..
		ACERACEAE						
5243	*Acer saccharinum*	Baltimore Co., Md.	10/54	l, s	−	0	+	..
5032	*Acer saccharum*	Sandisfield, Mass.	8/54	l, s	−	0	0	+++
5018	*Acer spicatum*	Sheffield, Mass.	8/54	l, s	−	0	0	+++
6119A	*Acer truncatum*	Chico, Calif.	10/56	l, fr	−	0	0	+++
6119B	*Acer truncatum*	Chico, Calif.	10/56	tw	−	0	0	+
		AIZOACEAE						
5284	*Carpobrotus edulis* (*Mesembryanthemum edule*)	St. Johns Co., Fla.	11/54	l, s	−	..	0	..
5495	*Sesuvium portulacastrum*	Monroe Co., Fla.	8/55	l, s	−	0	0	..
5727	*Sesuvium portulacastrum*	Pecos Co., Tex.	9/55	l, s, fl	−	0	0	..
		ALISMACEAE						
5400	*Sagittaria sp.*	Leon Co., Fla.	7/55	l, s, r, fl	−	0	+	..
		AMARANTHACEAE						
5685	*Amaranthus crassipes*	Brewster Co., Tex.	8/55	l, s, fl	−	0	0	..
5684	*Amaranthus palmeri*	Brewster Co., Tex.	8/55	l, s, r, fl	−	0	0	..
5114	*Amaranthus retroflexus*	Sussex Co., Del.	8/54	l, s, r, fl	−	0	0	..
6155	*Celosia argentea*	Baltimore Co., Md.	11/56	l, s, r, fl	−	0	0	0
5673	*Guilleminea densa* (*Brayulinea densa*)	Brewster Co., Tex.	8/55	l, s, fl	−	0	0	..
5055	*Iresine celosia*	Levy Co., Fla.	8/54	l, s, r, fl	−	0	0	0
5748	*Iresine heterophylla*	Jeff Davis Co., Tex.	9/55	l, s, fl	−	0	0	..
5635	*Tidestromia carnosa*	Brewster Co., Tex.	8/55	l, s, fl	−	0	0	..
5614	*Tidestromia lanuginosa*	Jeff Davis Co., Tex.	8/55	l, s	−	0	0	..
5662	*Tidestromia suffruticosa*	Brewster Co., Tex.	8/55	l, s, fl	−	0	0	..

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		AMARYLLIDACEAE						
5266	*Hymenocallis sp.*	Leon Co., Fla.	10/54	l, s, r	–	0	+	..
5173	*Manfreda sp.*	Glenn Dale, Md.	9/54	l, r	–	0	..	..
5174	*Manfreda sp.*	Glenn Dale, Md.	9/54	l, r	–	0	0	..
5175	*Manfreda sp.*	Glenn Dale, Md.	9/54	l, r	–	0	..	..
5176A	*Manfreda sp.*	Glenn Dale, Md.	9/54	l	–	0	..	..
5176B	*Manfreda sp.*	Glenn Dale, Md.	9/54	r	+	0	..	..
5177A	*Manfreda sp.*	Glenn Dale, Md.	9/54	l	–	0	..	..
5177B	*Manfreda sp.*	Glenn Dale, Md.	9/54	r	+	0	..	..
5178A	*Manfreda sp.*	Glenn Dale, Md.	9/54	l	–	0	..	..
5178B	*Manfreda sp.*	Glenn Dale, Md.	9/54	r	+	0	..	..
5179A	*Manfreda sp.*	Glenn Dale, Md.	9/54	l	–	0	..	..
5179B	*Manfreda sp.*	Glenn Dale, Md.	9/54	r	+	0	..	..
5180A	*Manfreda sp.*	Glenn Dale, Md.	9/54	l	–	0	..	..
5180B	*Manfreda sp.*	Glenn Dale, Md.	9/54	r	+	0	..	..
5181A	*Manfreda sp.*	Glenn Dale, Md.	9/54	l	–	0	..	..
5181B	*Manfreda sp.*	Glenn Dale, Md.	9/54	r	+	0	..	..
		ANACARDIACEAE						
5870	*Anacardium excelsum*	Atenas, Costa Rica	4/56	l, s, b	–	0	0	..
5520	*Mangifera indica*	Lee Co., Fla.	8/55	l, tw	–	0	0	..
6103A	*Pistacia Chinensis*	Chico, Calif.	10/56	l, tw	–	+	0	+++
6103B	*Pistacia Chinensis*	Chico, Calif.	10/56	fr	–	0	0	0
6118A	*Pistacia lentiscus*	Chico, Calif.	10/56	l	–	0	0	+++
6118B	*Pistacia lentiscus*	Chico, Calif.	10/56	tw	–	0	0	0
6123A	*Pistacia lentiscus*	Chico, Calif.	10/56	l	–	0	0	+++
6123B	*Pistacia lentiscus*	Chico, Calif.	10/56	tw	–	0	0	0
6127A	*Pistacia terebinthus*	Chico, Calif.	10/56	l	–	0	0	+++
6127B	*Pistacia terebinthus*	Chico, Calif.	10/56	fr	–	+	0	0
6127C	*Pistacia terebinthus*	Chico, Calif.	10/56	wd	–	0	0	0
6121A	*Pistacia vera*	Chico, Calif.	10/56	fr	–	0	0	0
6121B	*Pistacia vera*	Chico, Calif.	10/56	tw	–	0	0	0
5278	*Rhus copallina*	Leon Co., Fla.	11/54	l, s, r, fr	–	0	0	..
6043-OA	*Rhus copallina*	Woodstown, N. J.	10/57	sd	–	++	0	..
6043-OB	*Rhus copallina*	Woodstown, N. J.	10/57	chaff & seed residue	–	0	0	+++
5567	*Rhus microphylla*	Brewster Co., Tex.	8/55	l, tw	–	0	0	..
5238	*Rhus radicans*	Baltimore Co., Md.	10/54	l, s, fr	–	0	0	0
5538	*Rhus virens*	Brewster Co., Tex.	8/55	l, s	–	+	0	..
6181A	*Schinopsis lorentzii*	C. G. F.	11/57	l	–	0	0	+++
6181B	*Schinopsis lorentzii*	C. G. F.	11/57	s	–	0	0	0
5514	*Schinus terebinthifolius*	Collier Co., Fla.	8/55	l, tw	–	0	0	..
		ANNONACEAE						
3030	*Annona bullata*	C. G. F.	9/52	l	..	..	+	..
6067A	*Annona purpurea*	C. G. F.	8/56	l	–	+	0	0
6067B	*Annona purpurea*	C. G. F.	8/56	tw	–	0	0	0

5395A	*Asimina angustifolia*	Leon Co., Fla.	7/55	l	–	+	0	..
5395B	*Asimina angustifolia*	Leon Co., Fla.	7/55	tw	–	0	0	..
5069	*Asimina obovata*	Citrus Co., Fla.	8/54	l, s	–	0	0	0
5900	*Guatteria sp.*	Guanacaste, Costa Rica	5/56	l, s	–	0	0	..
		APOCYNACEAE						
6340	*Alafia sp.*	Beltsville, Md.	6/57	r	–	++	0	..
6341	*Alafia sp.*	Beltsville, Md.	6/57	sd	–	0	0	..
6342	*Alafia sp.*	Beltsville, Md.	6/57	fr	–	0	0	..
6343	*Alafia sp.*	Beltsville, Md.	6/57	sd	–	0	0	..
5436	*Allamanda cathartica*	Highlands Co., Fla.	8/55	l, tw	–	0	0	..
6086A	*Alstonia macrophylla*	C. G. F.	9/56	l	–	0	+	0
6086B	*Alstonia macrophylla*	C. G. F.	9/56	tw	–	0	+	0
6287	*Amsonia standleyi*	Candellaria, Tex.	4/57	l, s, fl, fr	–	0	0	..
6288	*Amsonia standleyi*	Candellaria, Tex.	4/57	r	–	0	0	..
5564	*Apocynum androsaemifolium*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..
5871-O	*Apocynum cannabinum*	Montgomery Co., Pa.	7/56	l	–	0	0	..
5367	*Cerebera odollam*	Okinawa	6/55	l, s	–	0	0	..
5510	*Echites umbellata*	Monroe Co., Fla.	8/55	w	–	0	0	..
6088	*Funtumia elastica*	C. G. F.	9/56	l, tw, fr	–	0	0	0
6012	*Kickxia elatine*	Va.	7/56	l, s, r, fl	–	0	0	..
5590	*Macrosiphonia macrosiphon*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0	..
6310	*Macrosiphonia sp.*	Sonora, Mexico	5/57	l. s, r	–	+	0	..
6374	*Marsdenia edulis*	Mexico	6/57	sd	–	..	..	..
6027-O	*Nerium oleander*	Charleston, S. C.	10/57	l, tw	–	0	0	..
5306A	*Ochrosia elliptica*	C. G. F.	11/54	l	–	0	+	0
5306B	*Ochrosia elliptica*	C. G. F.	11/54	tw	–	0	+	0
5306C	*Ochrosia elliptica*	C. G. F.	11/54	fr	–	0	0	0
6324	*Stemmadenia palmeri*	Sonora, Mexico	5/57	tw	–	0	+	..
6354	*Strophanthus amboensis*	Beltsville, Md.	6/57	sd	–	0	0	..
6334	*S. ophanthus congoensis*	Beltsville, Md.	6/57	fr	–	0	0	..
6337	*Strophanthus congoensis*	Beltsville, Md.	6/57	sd	–	0	0	..
6346	*Strophanthus courmontii*	Beltsville, Md.	6/57	sd	–	0	0	..
6350	*Strophanthus ecaudatus*	Beltsville, Md.	6/57	sd	–	0	0	..
6352	*Strophanthus gerrardii*	Beltsville, Md.	6/57	sd	–	0	0	..
6348	*Strophanthus grandiflorus*	Beltsville, Md.	6/57	sd	–	0	..	..
6338	*Strophanthus gratus*	Beltsville, Md.	6/57	sd	–	0	0	..
6356	*Strophanthus gratus*	Beltsville, Md.	6/57	sd, pod	–	0	0	..
6339	*Strophanthus hispidus*	Beltsville, Md.	6/57	sd	–	++	0	..
6349	*Strophanthus intermedius*	Beltsville, Md.	6/57	sd	–	0	0	..
6333	*Strophanthus mortehani*	Beltsville, Md.	6/57	rb	–	0	0	..
6356A	*Strophanthus periploca*	Beltsville, Md.	6/57	sd, pod	–	0	0	..
6353	*Strophanthus petersianus*	Beltsville, Md.	6/57	sd	–	0	0	..
6347	*Strophanthus preussii*	Beltsville, Md.	6/57	sd	+	0	..	..
6336	*Strophanthus sarmentosus*	Beltsville, Md.	6/57	sd	–	0	0	..
6351	*Strophanthus wightianus*	Beltsville, Md.	6/57	sd	–	0	0	..
6335	*Strophanthus sp.*	Beltsville, Md.	6/57	fr	–	0	0	..
6344	*Strophanthus sp.*	Beltsville, Md.	6/57	sd	–	0	0	..
6345	*Strophanthus sp.*	Beltsville, Md.	6/57	sd, pod	–	0	0	..
6355	*Strophanthus sp.*	Beltsville, Md.	6/57	sd, pod	–	0	0	..

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		APOCYNACEAE						
6357	*Strophanthus sp.*	Beltsville, Md.	6/57	sd	−	0	0	..
6358	*Strophanthus sp.*	Beltsville, Md.	6/57	sd	−	0	0	..
6359	*Strophanthus sp.*	Beltsville, Md.	6/57	sd	−	0	0	..
5490	*Urechites lutea*	Monroe Co., Fla.	8/55	l, tw	−	0	0	..
5758	*Vinca minor*	Brewster Co., Tex.	10/55	l, s, fl	−	0	0	..
5905-O	*Vinca minor*	Montgomery Co., Pa.	8/56	l	−	0	..	..
		AQUIFOLIACEAE						
5019	*Ilex laevigata*	Sheffield, Mass.	8/54	l, s, fr	−	0	0	+
5253	*Ilex myrtifolia*	Calhoun Co., Fla.	10/54	l, s, fr	−	0	0	..
6171	*Ilex vomitoria*	...	12/56	l	−	0	0	++
		ARACEAE						
5598	*Amorphophallus bulbifer*	C. G. F.	8/55	l, s	−	0	0	..
5261	*Colocasia antiquorum*	Leon Co., Fla.	10/54	l, s, r	−	0	0	..
5263	*Colocasia odorata*	Leon Co., Fla.	10/54	l, s	−	0	0	..
5262	*Colocasia odorata*	Leon Co., Fla.	10/54	l, s	−	0	0	..
6194	*Montrichardia arborescens*	Dutch Guiana	1/57	sd	−	0	0	0
6282	*Philodendron sp.*	Mexico	4/57	s	+	0	0	..
5449	*Pistia stratiotes*	Palm Beach Co., Fla.	8/55	w	−	0	0	..
		ARALIACEAE						
6413	*Gilibertia arborea*	Chiapas, Mexico	8/57	l, s	−	0	0	..
5903-O	*Hedera helix*	Montgomery Co., Pa.	8/56	l	−	0	0	..
		ASCLEPIADACEAE						
5542	*Asclepias brachystephana*	Jeff Davis Co., Tex.	8/55	l, s, r, fr	−	0	0	..
6098	*Asclepias brachystephana*	Brewster Co., Tex.	10/56	l, s	−	0	0	0
5548	*Asclepias capricornu*	Jeff Davis Co., Tex.	8/55	l, s, r	−	0	0	..
5546	*Asclepias curassavica*	Palm Beach Co., Fla.	8/55	w	−	0	0	..
5541	*Asclepias galioides*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	−	0	++	..
5878-OA	*Asclepias incarnata*	Montgomery Co., Pa.	8/56	l	−	0	0	..
5878-OB	*Asclepias incarnata*	Montgomery Co., Pa.	8/56	fl	−	+	0	..
5878-OC	*Asclepias incarnata*	Montgomery Co., Pa.	8/56	r	−	0	0	..
5879-O	*Asclepias incarnata* var. *pulchra* (*Asclepias pulchra*)	Montgomery Co., Pa.	8/56	l, fl, r	−	0	0	..
5906-O	*Asclepias incarnata* var. *pulchra* (*Asclepias pulchra*)	Montgomery Co., Pa.	8/56	l, r, pods	−	0	..	..
5761	*Asclepias latifolia*	Jeff Davis Co., Tex.	8/55	w	−	0	0	..
6421	*Asclepias linearis*	Sonora, Mexico	8/57	r	−	0	0	..
5428	*Asclepias perennis*	Dixie Co., Fla.	8/55	w	−	0	0	..
6139A	*Asclepias subverticillata*	Culbersen Co., Tex.	10/56	l	−	+	0	0
6139B	*Asclepias subverticillata*	Culbersen Co., Tex.	10/56	s	−	0	0	0
5811	*Asclepias subverticillata*	Jeff Davis Co., Tex.	12/55	l, s	−	0	+	..
5877-O	*Asclepias syriaca*	Montgomery Co., Pa.	8/56	l, s, r	−	0	0	..
5917-OA	*Asclepias syriaca*	Philadelphia, Pa.	9/56	sd	−	0	0	..
5917-OB	*Asclepias syriaca*	Philadelphia, Pa.	9/56	r	−	0	++	..
5920-O	*Asclepias syriaca*	Philadelphia, Pa.	10/56	fr, r	−	0	..	..
5894-O	*Asclepias tuberosa*	Perry Co., Pa.	8/56	l, r	−	0	0	..

5094	*Asclepias sp.*	Polk Co., Fla.	8/54	l, s, r, fl	–	+	0	..
5519	*Cryptostegia grandiflora*	Lee Co., Fla.	8/55	l, tw	–	0	0	..
5169	*Morrenia odorata*	Glenn Dale, Md.	9/54	l, s	–	0	0	..
		BATIDACEAE						
5493	*Batis maritima*	Monroe Co., Fla.	8/55	l, tw	–	0	0	..
		BERBERIDACEAE						
5030	*Berberis vulgaris*	Berkshire Co., Mass.	8/54	l, s, fr	–	0	++	0
5925	*Jeffersonia diphylla*	Washington Co., Md.	5/56	l, s, r	–	0	0	..
6321	*Odostemon longipes*	Sonora, Mexico	5/57	wd	–	0	++	..
		BETULACEAE						
5111	*Alnus maritima*	Sussex Co., Del.	8/54	l, s, fr	–	0	0	..
5017	*Corylus cornuta*	Sheffield, Mass.	8/54	l, s	–	0	0	++
		BIGNONIACEAE						
6129	*Catalpa hybrida*	Chico, Calif.	10/56	fr	–	0	0	0
5415	*Doxantha unguis-cati*	Leon Co., Fla.	7/55	w	–	0	0	..
6201A	*Jacaranda acutifolia*	C. G. F.	1/57	l, s	–	++	0	0
6201B	*Jacaranda acutifolia*	C. G. F.	1/57	tw	–	0	0	0
6201C	*Jacaranda acutifolia*	C. G. F.	1/57	pod	–	0	0	0
6201D	*Jacaranda acutifolia*	C. G. F.	1/57	sd	–	0	0	0
6168	*Spathodea nilotica*	C. G. F.	12/56	l, tw	–	0	0	0
6077	*Tecoma gaudichaudii*	C. G. F.	9/56	l, tw	–	0	0	0
		BOMBACACEAE						
6417	*Ochroma lagopus*	Chiapas, Mexico	8/57	fr, s	–	0	0	..
		BORAGINACEAE						
5508	*Bourreria ovata*	Monroe Co., Fla.	8/55	l, tw, fr	–	0	0	..
5657	*Coldenia greggii*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
5655	*Coldenia hispidissima*	Brewster Co., Tex.	8/55	l, s	–	0	0	..
5629	*Coldenia Mexicana*	Brewster Co., Tex.	8/55	l, s	–	0	0	..
5869	*Cordia alliodora*	La Carita, Costa Rica	4/56	l, s	–	0	0	..
5631	*Heliotropium confertifolium*	Brewster Co., Tex.	8/55	l, s	–	0	0	..
5721	*Heliotropium convolvulaceum*	Ward Co., Tex.	9/55	l, s, r, fl	–	0	0	..
5499	*Heliotropium curassavicum*	Monroe Co., Fla.	8/55	w	–	0	0	..
5452	*Heliotropium leavenworthii*	Broward Co., Fla.	8/55	l, s, r, fl	–	0	0	..
6034	*Lappula Virginiana*	Washington Co., Md.	7/56	l, s, r	–	0	0	..
5927	*Mertensia Virginica*	Washington Co., Md.	5/56	l, s	–	0	0	..
5997	*Myosotis scorpioides*	Winsted, Conn.	6/56	l, s, fl	–	0	+	..
5454	*Tournefortia gnaphalodes*	Broward Co., Fla.	8/55	l, tw, fr	–	0	0	..
5630	*Tournefortia monclovana*	Brewster Co., Tex.	8/55	l, s	–	0	0	..
		BROMELIACEAE						
5189	*Aechmea Mexicana*	C. G. F.	8/55	l, s	–	0	0	..
		BURSERACEAE						
5482	*Bursera simaruba*	Monroe Co., Fla.	8/55	l, tw, fr	–	0	0	..
		BUXACEAE						
6061	*Buxus harlandii*	Chico, Calif.	8/56	l, s	–	0	+	0
5293	*Simmondsia Chinensis*	Aguangua, Calif.	11/54	pod	+	..	..	..

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		BUXACEAE						
6375A	*Simmondsia Chinensis*	Murrieta, Calif.	6/57	sd	–	0	0	+
6375B	*Simmondsia Chinensis*	Murrieta, Calif.	6/57	pod	–	0	0	0
		CACTACEAE						
6019-O	*Cereus parviflorus*	Kingston, Jamaica, B. W. I.	10/57	l	–	0	..	..
5950-O	*Cereus Peruvianus (Cereus cf. swartzii)*	May-Pen, Jamaica, B. W. I.	5/57	l	–	0	..	0
5958-O	*Cereus Peruvianus (Cereus cf. swartzii)*	May-Pen, Jamaica, B. W. I.	6/57	l	–	0	..	..
6022-O	*Cereus triangularis*	Kingston, Jamaica, B. W. I.	10/57	l	–	0	..	..
6097	*Echinocereus dasyacanthus*	Brewster Co., Tex.	10/56	w	–	0	0	0
5916-O	*Lophophora williamsii*	Weslaco, Tex.	9/56	t	–	0	..	..
5951-O	*Melocactus communis*	May-Pen, Jamaica, B. W. I.	5/57	l	–	0	..	0
6016-O	*Nopalea coccinellifera*	Kingston, Jamaica, B. W. I.	10/57	l	–	0	..	..
5949-O	*Opuntia dillenii*	May-Pen, Jamaica, B. W. I.	5/57	l	–	0	..	0
6286	*Opuntia grahamii*	Candellaria, Tex.	4/57	w	–	0	0	..
5941-O	*Opuntia spinosissima*	Clarendon, Jamaica, B. W. I.	5/57	l	–	0	..	0
6023-O	*Opuntia tuna*	Kingston, Jamaica, B. W. I.	10/57	l	–	0	..	..
5957-O	*Opuntia tuna*	May-Pen, Jamaica, B. W. I.	6/57	l	–	0	..	..
		CALLITRICHACEAE						
6048	*Callitriche heterophylla*	Baltimore Co., Md.	8/56	w	–	0	0	..
		CAMPANULACEAE						
5106	*Lobelia puberula*	Talbot Co., Md.	8/54	l, s, r, fl	–	0	+	..
5116	*Lobelia siphilitica*	Baltimore Co., Md.	8/54	l, s, r, fl	–	0	++	..
		CANNACEAE						
5443	*Canna indica*	Palm Beach, Fla.	8/55	l, s	–	0	0	..
		CAPPARIDACEAE						
6418	*Capparis cf. indica*	Chiapas, Mexico	8/57	l, s	–	0	0	..
6411	*Capparis cf. oxysepala*	Chiapas, Mexico	8/57	l, s	–	0	0	..
5431	*Polanisia tenuifolia*	Lake Co., Fla.	8/55	w	–	0	0	..
		CAPRIFOLIACEAE						
5036	*Sambucus pubens*	Winsted, Conn.	8/54	l, s	–	0	0	0
5086	*Sambucus simpsonii*	Pinellas Co., Fla.	8/54	l, s, fr	–	0	0	..
5020	*Viburnum alnifolium*	Berkshire Co., Mass.	8/54	l, s	–	0	0	0
5040	*Viburnum dentatum*	Torrington, Conn.	8/54	s, fr	–	0	0	0
3840	*Viburnum nudum*	Wake Co., N. C.	4/54	l, tw, fl	..	..	+	..
6028-O	*Viburnum tinus*	Charleston, S. C.	10/57	l, s	–	0	0	..
		CARYOPHYLLACEAE						
5951	*Agrostemma githago*	Anne Arundel Co., Md.	5/56	l, s, r, fl	–	0	0	..
5943	*Cerastium viscosum*	Baltimore Co., Md.	5/56	l, s, r	–	0	0	..
5929	*Holosteum umbellatum*	Washington Co., Md.	5/56	l, s, r, fl	–	0	0	..
5950	*Scleranthus annuus*	Anne Arundel Co., Md.	5/56	l, s, r	–	0	0	..
5119	*Stellaria aquatica*	York Co., Pa.	8/54	l, s, r, fl	–	0	0	..

		CASUARINACEAE						
5076	*Casuarina equisetifolia*	Pinellas Co., Fla.	8/54	l, s, fr	–	0	0	0
		CELASTRACEAE						
5919-O	*Celastrus orbiculatus*	Montgomery Co., Pa.	9/56	l	–	0	0	..
5921-O	*Euonymus atropurpureus*	Montgomery Co., Pa.	10/56	fr	–	..	0	..
		CHENOPODIACEAE						
5719	*Allenrolfea occidentalis*	Pecos Co., Tex.	9/55	l, s	–	0	0	..
5594	*Atriplex canescens*	Jeff Davis Co., Tex.	8/55	l, tw, fr	–	0	0	..
5633	*Atriplex obovata*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
5740	*Atriplex sp.*	Pecos Co., Tex.	9/55	l, s, fl	–	0	0	..
5571	*Atriplex sp.*	Brewster Co., Tex.	8/55	l, s, r	–	0	0	..
5650	*Atriplex sp.*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5716	*Chenopodium ambrosioides*	Terrell Co., Tex.	9/55	l, s, r, fl	–	0	0	..
5577	*Chenopodium incanum*	Jeff Davis Co., Tex.	8/55	l, fl, tw	–	0	0	..
5554	*Chenopodium sp.*	Jeff Davis Co., Tex.	8/55	l, s, r	–	0	0	..
5406	*Chenopodium sp.*	Leon Co., Fla.	7/55	l, s, fl	–	0	0	..
5402	*Chenopodium sp.*	Leon Co., Fla.	7/55	l, s, fl	–	0	0	..
5744	*Cycloloma atriplicifolium*	Ward Co., Tex.	9/55	l, s, r, fl	–	0	0	..
5498	*Salicornia Virginica*	Monroe Co., Fla.	8/55	l, s, r	–	0	0	..
5674	*Salsola pestifer*	Brewster Co., Tex.	8/55	l, s, r	–	0	0	..
5500	*Suaeda linearis*	Monroe Co., Fla.	8/55	l, s, r, fr	–	0	0	..
5663	*Suaeda suffrutescens* (*Dondia suffrutescens*)	Brewster Co., Tex.	8/55	l, s	–	0	0	..
		CISTACEAE						
6022	*Helianthemum Canadense*	Accomac Co., Va.	7/56	l, s, r	–	0	0	..
		CLADONIACEAE (LICHENES)						
6031	*Cladonia submitis*	Aiken, S. C.	7/56	l, s	–	0	0	..
		CLETHRACEAE						
5899-O	*Clethra alnifolia*	Burlington Co., N. J.	8/56	l, fl	–	0	0	..
		COMBRETACEAE						
5465	*Conocarpus erectus*	Dade Co., Fla.	8/55	l, tw	–	0	0	..
5070	*Laguncularia racemosa*	Pinellas Co., Fla.	8/54	l, s, fr	–	0	0	+++
		COMMELINACEAE						
5370	*Tradescantia fluminensis*	Leon Co., Fla.	7/55	l, s, r	–	0	0	..
		COMPOSITAE						
5223	*Actinospermum angustifolium*	Franklin Co., Fla.	10/54	l, s, r, fl	–	+	0	..
5496	*Ambrosia hispida*	Monroe Co., Fla.	8/55	l, s, r	–	0	+	..
5693	*Ambrosia trifida*	Val Verde Co., Tex.	9/55	l, s, fl	–	0	0	..
6083	*Arnica whitneyi* (*Arnica cordifolia*)	Alberta, Canada	9/56	l, s, r, fl	–	0	0	0
5113	*Artemisia caudata*	Sussex Co., Del.	8/54	l, s	–	0	0	..
5753	*Artemisia filifolia*	Ward Co., Tex.	9/55	l, s, fl	–	0	0	..
5624	*Artemisia Mexicana*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..
5271	*Aster Carolinianus*	Wakulla Co., Fla.	10/54	l, s, fl	–	0	0	..
5034	*Aster cf. acuminatus*	Winsted, Conn.	8/54	l, s, fr	–	0	0	0
5273	*Aster exilis*	Wakulla Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5239	*Aster Novae Angliae*	Baltimore Co., Md.	10/54	l, s, fl	–	0	0	..
5746	*Aster tenacetifolius*	Pecos Co., Tex.	9/55	l, s, fl	–	0	0	..

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		COMPOSITAE						
5079	*Aster tenuifolius*	Pinellas Co., Fla.	8/54	l, s, r	–	0	0	0
5199	*Aster undulatus*	Allegany Co., Md.	9/54	l, s, r, fl	–	0	0	..
5747	*Aster sp.*	Reeves Co., Tex.	9/55	l, s, fl	–	0	0	..
5258	*Aster sp.*	Calhoun Co., Fla.	10/54	l, s, r	–	0	0	..
5272	*Baccharis glomeruliflora*	Wakulla Co., Fla.	10/54	l, s, fl	–	0	0	..
5574	*Baccharis glutinosa*	Brewster Co., Tex.	8/55	l, tw, fl	–	0	+	..
5875	*Baccharis halimifolia*	Ocean Co., N. J.	8/56	l, s	–	0	0	..
5737	*Baccharis havardii*	Pecos Co., Tex.	9/55	l, tw	–	0	0	..
5661	*Bahia absinthifolia*	Brewster Co., Tex.	8/55	l, s, fl	–	0	+ +	..
5649	*Bahia pedata*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
5604	*Baileya multiradiata*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5611	*Berlandiera lyrata*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..
5097	*Berlandiera subacaulis*	Orange Co., Fla.	8/54	l, s, r	–	0	0	..
5123	*Bidens comosa*	York Co., Pa.	8/54	l, s, r, fl	–	0	0	..
5103	*Bidens coronata*	Anne Arundel Co., Md.	8/54	l, s, r, fl	–	0	0	..
5121	*Bidens frondosa*	York Co., Pa.	8/54	l, s, r, fl	–	+	0	..
5209	*Bidens laevis*	Baltimore Co., Md.	9/54	l, s, r, fl	–	0	0	..
5610	*Bidens leptocephala*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..
5044	*Bidens mitis*	Taylor Co., Fla.	8/54	l, s	–	0	0	0
5234	*Bidens nashii*	Jefferson Co., Fla.	10/54	l, s, r, fl	–	+	0	..
5883-O	*Bidens sp.*	Lester, Pa.	8/56	w, r	–	0	0	..
5157	*Boltonia asteroides*	Windsor, N. C.	9/54	l, s, r, fl	–	0	0	..
5470	*Borrichia arborescens*	Monroe Co., Fla.	8/55	l, tw, fr	–	0	0	..
5559	*Brickellia laciniata*	Brewster Co., Tex.	8/55	l, s, tw	–	0	+	..
5054	*Cacalia sulcata*	Levy Co., Fla.	8/54	l, s, r, fl	–	0	+ +	0
5397	*Calyptocarpus vialis*	Leon Co., Fla.	7/55	w	+	0	0	..
5046	*Carphephorus corymbosus*	Taylor Co., Fla.	8/54	l, s, fl, r	–	0	+	0
5762	*Chrysoma pauciflosculosa*	Gulf Co., Fla.	10/55	l, s, fl	–	0	0	..
5765	*Chrysopsis cruiseana*	Gulf Co., Fla.	10/55	l, s, r, fl	–	+	0	..
5213	*Chrysopsis flexuosa*	Leon Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5093	*Chrysopsis graminifolia* var. *latifolia*	Polk Co., Fla.	8/54	l, s	–	0	0	..
5250	*Chrysopsis hyssopifolia*	Leon Co., Fla.	10/54	l, s, fl	–	0	0	..
5764	*Chrysopsis mixta*	Gulf Co., Fla.	10/55	l, s, r, fl	–	+	0	..
5435	*Chrysopsis subulata*	Polk Co., Fla.	8/55	l, s, r, fl	–	+ +	0	..
5738	*Chrysothamnus sp.*	Ward Co., Tex.	9/55	l, tw, fl	–	0	0	..
5246	*Cirsium Carolinianum*	Liberty Co., Fla.	10/54	l, s, fl	–	0	0	..
5425	*Cirsium lecontei*	Jackson Co., Miss.	8/55	w	–	0	0	..
5053	*Cirsium nuttallii*	Levy Co., Fla.	8/54	l, s, fl	–	0	0	0
5543	*Cirsium undulatum*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5041	*Cirsium cf. vittatum*	Taylor Co., Fla.	8/54	l, s, fl, r	–	0	0	0
5613	*Conyza coulteri*	Jeff Davis Co., Tex.	8/55	l, s, tw	–	0	0	..
6140	*Conyza coulteri*	Pecos Co., Tex.	10/56	l, s, fl	–	+ +	0	0
5042	*Coreopsis leavenworthii*	Taylor Co., Fla.	8/54	l, s, fl, r	–	0	0	0
5429	*Coreopsis leavenworthii* var. *curtissii*	Dixie Co., Fla.	8/55	w	–	0	0	..
5526	*Coreopsis leavenworthii* var. *lewtonii*	Manatee Co., Fla.	8/55	l, s, r, fl	–	0	0	..

6156	*Cosmos bipinnatus*	Baltimore, Md.	11/56	l, s, r, fl	–	0	0	0	
5656	*Dyssodia acerosa*	Brewster Co., Tex.	8/55	w	–	0	0		
5695	*Dyssodia pentachaeta*	Pecos Co., Tex.	9/55	l, s, fl	–	0	0		
5703	*Dyssodia polychaeta*	Brewster Co., Tex.	9/55	l, s, fl	–	0	+		
5734	*Dyssodia sp.*	Jeff Davis Co., Tex.	9/55	l, s, r, fl	–	0	0		
5561	*Engelmannia pinnatifida*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0		
5888-O	*Erechtites hieracifolia*	Perry Co., Pa.	8/56	l, fl, r	–	0	0		
5405	*Erigeron bonariensis*	Leon Co., Fla.	7/55	w	–	0	0		
5741	*Erigeron Canadensis*	Reeves Co., Tex.	9/55	l, s, r, fl	–	0	0		
5690	*Eupatorium*	Val Verde Co., Tex.	9/55	l, s, fl	–	0	+		
5276	*Eupatorium aromaticum*	Leon Co., Fla.	11/54	l, s, fl	–	0	0		
5002	*Eupatorium coelestinum*	Leon Co., Fla.	8/54	l, s, r	–	0	0	0	
5028	*Eupatorium dubium*	New Boston, Mass.	8/54	l, s, fl, r	–	0	0	0	
5277	*Eupatorium incarnatum*	Leon Co., Fla.	11/54	l, s, r, fl	–	0	0		
5280	*Eupatorium jucundum*	St. Johns Co., Fla.	11/54	l, s, r, fl	–	0	0		
5166	*Eupatorium leptophyllum*	Leon Co., Fla.	9/54	l, s, r, fl	–	0	+		
5279	*Eupatorium leucolepis*	St. Johns Co., Fla.	11/54	l, s, r, fl	–	0	0		
5225	*Eupatorium perfoliatum*	Leon Co., Fla.	10/54	l, s, r, fl	–	0	0		
5903	*Eupatorium pittieri*	Guanacaste, Costa Rica	5/56	l, s	–	0	0		
5014	*Eupatorium rugosum*	Colebrook, Conn.	8/54	l, s, fl, r	–	0	0	0	
5484	*Flaveria bidentis*	Monroe Co., Fla.	8/55	l, tw	–	0	0		
5236	*Flaveria linearis*	Wakulla Co., Fla.	10/54	l, s, r, fr	–	++	0		
5743	*Flaveria trinervia*	Pecos Co., Tex.	9/55	l, tw, fl	–	++	0		
5670	*Flourensia cernua*	Brewster Co., Tex.	8/55	w	–	0	+		
6141	*Flourensia cernua*	Brewster Co., Tex.	10/56	l, s, fl	–	+	+	0	
5551	*Franseria acanthicarpa*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	++		
5694	*Franseria sp.*	Val Verde Co., Tex.	9/55	l, s, fl	–	0	+		
5751	*Gaillardia amblyodon*	Reeves Co., Tex.	9/55	l, s, fl	–	0	+		
5750	*Gaillardia pulchella*	Ward Co., Tex.	9/55	l, s, fl	–	0	0		
5022	*Gnaphalium uliginosum*	Sandisfield, Mass.	8/54	l, s, r	–	0	0	0	
5732	*Grindelia sp.*	Pecos Co., Tex.	9/55	l, s, fl	–	0	0		
5645	*Gutierrezia sarothrae*	Brewster Co., Tex.	8/55	l, s, r, fl	–	+	0		
5654	*Gutierrezia sp.*	Brewster Co., Tex.	8/55	l, s, fl	–	0	+		
5659	*Haploesthes greggii*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0		
5720	*Haplopappus heterophyllus*	Pecos Co., Tex.	9/55	l, s, fl	–	0	0		
5235	*Helenium autumnale* var. *parviflorum*	Wakulla Co., Fla.	10/54	l, s, r, fl	–	0	0		
5536	*Helenium badium*	Jeff Davis Co., Tex.	8/55	w	–	0	+		
5686	*Helenium microcephalum*	Brewster Co., Tex.	8/55	l, s, fl	–	0	+		
5215	*Helianthus angustifolius*	Wakulla Co., Fla.	10/54	l, s, r, fl	–	0	0		
5683	*Helianthus annuus*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0		
5151	*Helianthus atrorubens*	Petersburg, Va.	9/54	l, s, r, fl	–	0	0		
5733	*Helianthus ciliaris*	Pecos Co., Tex.	9/55	l, s, r, fl	–	0	0		
5283	*Helianthus debilis*	St. Johns Co., Fla.	11/54	l, s, r, fl	–	0	0		
5072	*Helianthus Floridanus*	Pinellas Co., Fla.	8/54	l, s, r	–	+++	0	0	
5005	*Helianthus laevis*	Raritan, N. J.	8/54	l, s, fl	–	0	0	0	
5221	*Helianthus radula*	Wakulla Co., Fla.	10/54	l, s, r, fl	–	0	0		
5724	*Helianthus sp.*	Ward Co., Tex.	9/55	l, s, r, fl	–	0	0		
5893-O	*Helianthus sp.*	Newport Co., Pa.	8/56	l	–	0	0		
5730	*Heterotheca subaxillaris*	Ward Co., Tex.	9/55	l, s, fl	–	0	0		
5576	*Hymenoclea monogyra*	Brewster Co., Tex.	8/55	l, tw	–	0	0		

Accession No.	Species	Collection Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		COMPOSITAE						
5409	*Hypochoeris Brasiliensis*	Leon Co., Fla.	7/55	w	–	0	0	..
5658	*Iva ambrosiaefolia*	Brewster Co., Tex.	8/55	l, s	–	0	0	..
6021	*Krigia Virginica*	Accomack Co., Va.	7/56	l, s, fl	–	0	0	..
5882-O	*Lactuca Canadensis*	Delaware Co., Pa.	8/56	l, r	–	0	0	..
5957	*Lapsana communis*	King George Co., Va.	5/56	l, s, r, fl	–	0	0	..
5229	*Liatris elegans*	Jefferson Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5222	*Liatris gracilis*	Wakulla Co., Fla.	10/54	l, s, r, fl	–	0	+	..
5237	*Liatris graminifolia* var. *elegantula*	Leon Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5203	*Liatris scariosa*	Allegany Co., Md.	9/54	l, s, r, fl	–	0	0	..
5251	*Liatris spicata* var. *resinosa*	Calhoun Co., Fla.	10/54	l, s, fl, r	–	0	0	..
5587	*Lygodesmia sp.*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5504	*Melanthera parvifolia*	Monroe Co., Fla.	8/55	w	–	0	0	..
6326	*Montanoa rosei*	Sonora, Mexico	5/57	s	–	0	0	..
5566	*Parthenium incanum*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	+	..
5626	*Pectis angustifolia*	Brewster Co., Tex.	8/55	l, s, tw, fl	–	0	0	..
5588	*Perezia wrightii*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..
5664	*Perityle vaseyi*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
5528	*Phoebanthus grandiflorus*	Pinellas Co., Fla.	8/55	l, s, r, fl	–	0	0	..
5372	*Phoebanthus tenuifolius*	Liberty Co., Fla.	7/55	l, s, r, fl	–	0	0	..
6043	*Picris hieracioides*	Livingston Co., N. Y.	7/56	l, s, r, fl	–	0	0	..
5212	*Polypteris integrifolia*	Leon Co., Fla.	10/54	l, s, r, fl	–	0	+	..
5647	*Porophyllum scoparium*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5985	*Psilostrophe tagetina*	Brewster Co., Tex.	6/56	l, s, r, fl	–	0	0	..
5616	*Psilostrophe tagetina*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..
6013	*Pyrrhopappus Carolinianus*	Va.	7/56	l, s, r, fl	–	0	0	..
5558	*Ratibida columnifera* (*Ratibida columnaris*)	Brewster Co., Tex.	8/55	l, s, r, fr	–	0	0	..
5057	*Rudbeckia cf. heterophylla*	Levy Co., Fla.	8/54	l, s, r, fl	–	0	0	0
5869-O	*Rudbeckia hirta*	Montgomery Co., Pa.	7/56	l, fl	–	0	0	..
5892-O	*Rudbeckia laciniata*	Newport Co., Pa.	8/56	l, fl, r	–	0	0	..
5679	*Rudbeckia mohrii*	Calhoun Co., Fla.	9/55	l, s, r, fl	–	0	0	..
5718	*Schkuhria sp.*	Jeff Davis Co., Tex.	9/55	l, s, r	–	0	0	..
5607	*Senecio longilobus*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	+ +	..
5987	*Senecio longilobus*	Brewster Co., Tex.	6/56	l, s, r, fl	–	0	0	0
5704	*Simsia calva*	Terrell Co., Tex.	9/55	l, s, fl	–	0	0	..
5202	*Solidago caesia*	Allegany Co., Md.	9/54	l, s, r, fl	–	0	0	..
5874-O	*Solidago sempervirens*	Ocean Co., N. J.	8/56	w, r	–	0	0	..
5118	*Solidago speciosa*	Baltimore Co., Md.	8/54	l, s, r, fl	–	0	+	..
5873-OA	*Solidago sp.*	Montgomery Co., Pa.	8/56	l	–	0	..	..
5873-OB	*Solidago sp.*	Montgomery Co., Pa.	8/56	l, s	–	+	0	..
5873-OC	*Solidago sp.*	Montgomery Co., Pa.	8/56	basal l	–	0	0	..
5873-OD	*Solidago sp.*	Montgomery Co., Pa.	8/56	r	–	0	0	..
5872-O	*Solidago sp.*	Montgomery Co., Pa.	8/56	w, r	–	0	0	..
5233	*Solidago sp.*	Wakulla Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5201	*Solidago sp.*	Allegany Co., Md.	9/54	l, s, r, fl	–	0	0	..
5200	*Solidago sp.*	Allegany Co., Md.	9/54	l, s, r, fl	–	0	0	..

5260	*Spilanthes Americana*	Leon Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5424A	*Stokesia laevis*	Jackson Co., Miss.	8/55	l, s, fl	–	0	0	..
5424B	*Stokesia laevis*	Jackson Co., Miss.	8/55	r	–	0	++	..
5702	*Thelesperma longipes*	Terrell Co., Tex.	9/55	l, s, fl	–	0	0	..
5700	*Thelesperma simplicifolium*	Terrell Co., Tex.	9/55	l, s, fl	–	0	0	..
5723	*Thelesperma megapotamicum*	Ward Co., Tex.	9/55	l, s, r, fl	–	0	0	..
5586	*Trixis Californica*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	+	..
6039	*Tussilago farfara*	Dundee, N. Y.	7/56	w	–	0	0	..
5615	*Verbesina encelioides*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	+	..
5182	*Verbesina Virginica*	Wakulla Co., Fla.	9/54	l, s, r, fl	–	0	0	..
5712	*Vernonia lindheimeri*	Terrell Co., Tex.	9/55	l, s, fl	–	0	0	..
5553	*Vernonia marginata*	Brewster Co., Tex.	8/55	l, s	–	0	+	..
5891-O	*Vernonia noveboracensis*	Perry Co., Pa.	8/56	l, r	–	0	0	..
5256	*Vernonia sp.*	Liberty Co., Fla.	10/54	l, s, fl	–	0	0	..
5668	*Viguiera stenoloba*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
5736	*Viguiera sp.*	Jeff Davis Co., Tex.	9/55	l, s, fl	–	0	0	..
5687	*Xanthium sp.*	Brewster Co., Tex.	8/55	l, s, tw	–	0	0	..
5637	*Zexmenia brevifolia*	Brewster Co., Tex.	8/55	l, s, r	–	0	0	..
5569	*Zinnia grandiflora*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5628	*Zinnia pumila*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	0	..
		CONVOLVULACEAE						
5101	*Breweria grandiflora*	Lake Co., Fla.	8/54	l, s, r, fl	–	0	0	..
5618	*Convolvulus incanus*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..
6179	*Ipomoea bonariensis*	Savannah, Ga.	12/56	r	–	0	0	0
5401	*Ipomoea dissecta*	Leon Co., Fla.	7/55	l, s	–	0	0	..
5533	*Ipomoea stolonifera*	Pinellas Co., Fla.	8/55	l, s	–	0	0	..
5506	*Ipomoea tuba*	Monroe Co., Fla.	8/55	w	–	0	0	..
6373	*Ipomoea sp.*	Sonora, Mexico	6/57	t	–	0	0	..
5862	*Rapona sp.*	Costa Rica	4/56	l, s	–	0	0	..
		CRASSULACEAE						
5100	*Kalanchoe sp.*	Lake Co., Fla.	8/54	l, s, r,	–	0	0	..
		CRUCIFERAE						
5926	*Alliaria officinalis*	Washington Co., Md.	5/56	l, s	–	0	0	..
5980	*Alyssum alyssoides*	Washington Co., Md.	6/56	r, s, sd	–	0	0	..
5945	*Arabis laevigata*	Harvord Co., Md.	5/56	l, s, r	–	0	0	..
5907-O	*Brassica kaber*	Whilman Co., Wash.	9/56	fl	–	0	0	..
5978	*Brassica nitra*	Carroll Co., Md.	6/56	l, s, r	–	0	0	..
5908-O	*Camelina microcarpa*	Whitman Co., Wash.	9/56	fl, s	–	0	0	..
5909-O	*Capsella bursa-pastoris*	Whitman Co., Wash.	9/56	l, fl, s	–	0	0	..
6015	*Coronopus didymus* (*Carara didyma*)	Va.	7/56	l, s, r	–	0	0	..
5910-O	*Descurainia sophia*	Whitman Co., Wash.	9/56	l, tw	–	0	0	..
6035	*Draba ramosissima*	Washington Co., Md.	7/56	l, s, r, fl	–	0	0	..
5911-OA	*Erysimum occidentale*	Franklin Co., Wash.	8/56	l, tw	–	0	0	..
5911-OB	*Erysimum occidentale*	Franklin Co., Wash.	8/56	sd	–	+	0	..
5660	*Lepidium lasiocarpum*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	+	..
5912-O	*Lepidium perfoliatum*	Whitman Co., Wash.	9/56	l, tw, fl	–	0	0	..
5599	*Lepidium sordidum*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	+	..
5643	*Nerisyrenia camporum*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		CRUCIFERAE						
5129	*Raphanus sp.*	York Co., Pa.	8/54	l, s, r, fl	–	0	0	..
5552	*Rorippa officinale* (*Rorippa nasturtium-aquaticum*)	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..
5998	*Sisymbrium* (*Norta*) *altissimum*	Winsted, Conn.	6/56	l, s, fl	–	0	0	..
5913-O	*Sisymbrium altissimum*	Pullman, Wash.	9/56	l, tw	–	0	0	..
5928	*Thlaspi arvense*	Washington Co., Md.	5/56	l, s, r, fl	–	0	0	..
5914-O	*Thlaspi arvense*	Whitman Co., Wash.	9/56	tw, fl, sd	–	0	0	..
5381	*Warea amplexifolia*	Liberty Co., Fla.	7/55	w	–	0	0	..
		CUCURBITACEAE						
5696	*Apodanthera undulata*	Jeff Davis Co., Tex.	9/55	l, s	–	0	0	..
5672	*Cucurbita foetidissima*	Brewster Co., Tex.	8/55	l, s	–	0	0	..
5547	*Cucurbita foetidissima*	Brewster Co., Tex.	8/55	fr	–	0	0	..
5007	*Echinocystis lobata*	Lincolndale, N. Y.	8/54	l, s, fl, fr	–	0	+	0
5388	*Melothria pendula*	Leon Co., Fla.	7/55	w	–	0	0	..
5532	*Momordica charantia*	Pinellas Co., Fla.	8/55	l, s	–	0	0	..
		CYCADACEAE						
5268	*Cycas revoluta*	Leon Co., Fla.	10/54	l, s	–	0	0	..
5534	*Zamia integrifolia*	Pinellas Co., Fla.	8/55	l, s, r	–	0	0	..
		CYPERACEAE						
5513	*Cyperus ligularis*	Collier Co., Fla.	8/55	l, s, r, fr	–	0	0	..
5394	*Cyperus ovularis*	Leon Co., Fla.	7/55	w	–	0	0	..
5416	*Cyperus polystachyos* var. *texensis*	Leon Co., Fla.	8/55	w	–	+	0	..
6068	*Dulichium arundinaceum*	Charles Co., Md.	8/56	l, r	–	0	0	0
5067	*Eleocharis cellulosa*	Levy Co., Fla.	8/54	l, s, r, fl	–	0	0	0
5396	*Psilocarya scirpoides*	Leon Co., Fla.	7/55	w	–	0	0	..
5156	*Rhynchospora corniculata*	Windsor, N. C.	9/54	l, r, fl	–	0	0	..
		CYRILLACEAE						
5218	*Cyrilla paryifolia* (*Cyrilla parvifolia*)	Wakulla Co., Fla.	10/54	l, s, fr	–	0	0	..
		DIAPENSIACEAE						
5993	*Pyxidanthera barbulata*	Atsion, N. J.	6/56	l, s	–	0	0	..
		DIPSACACEAE						
5994	*Succisa sp.*	Batam, Conn.	6/56	l, s, r, fl	–	0	0	..
		ELAEAGNACEAE						
6046	*Shepherdia Canadensis*	Livingston Falls, Alberta, Canada	8/56	l, s	–	0	0	..
		EQUISETACEAE						
5038	*Equisetum sylvaticum*	Torrington, Conn.	8/54	l, s	–	0	0	0
		ERICACEAE						
6117A	*Arbutus andrachne*	Chico, Calif.	10/56	l, s	–	0	0	0
6117B	*Arbutus andrachne*	Chico, Calif.	10/56	tw	–	0	0	++
6002	*Gaultheria procumbens*	Wingsted, Conn.	6/56	l, s, r	–	0	0	..
6082	*Ledum Groenlandicum*	Livingston Falls, Alberta, Canada	9/56	l, tw	–	++	0	+++

5275	*Rhododendron nudiflorum*	Frederick Co., Md.	10/54	l, s, tw	–	0	0	..
		ERYTHROXYLACEAE						
6149A	*Erythoxylon coca*	C. G. F.	10/56	l	–	0	+	0
6149B	*Erythoxylon coca*	C. G. F.	10/56	fr	–	0	0	0
6149C	*Erythoxylon coca*	C. G. F.	10/56	tw	–	0	0	++
		EUPHORBIACEAE						
5390	*Acalypha gracilens*	Leon Co., Fla.	7/55	w	–	0	0	..
5185	*Acalypha ostryaefolia*	Leon Co., Fla.	9/54	l, s, r, fl	–	0	0	..
5710	*Acalypha radians*	Terrell Co., Tex.	9/55	w	–	0	0	..
5437	*Aleurites moluccana*	Highlands Co., Fla.	8/55	fr, tw	–	0	0	..
5380	*Croton argyranthemus*	Liberty Co., Fla.	7/55	w	–	0	0	..
5427	*Croton capitatus*	Jackson Co., Miss.	8/55	w	–	0	0	..
5537	*Croton corymbulosus*	Jeff Davis Co., Tex.	8/55	l, s, r, fr	–	0	0	..
5636	*Croton leucophyllus*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
5501	*Croton linearis*	Monroe Co., Fla.	8/55	l, s, r	–	0	++	..
5699	*Croton monanthogynus*	Terrell Co., Tex.	9/55	l, s, r, fl	–	0	0	..
5646	*Croton Neo Mexicanus*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5728	*Croton Texensis*	Ward Co., Tex.	9/55	l, s, r, fl	–	++	0	..
5045	*Croton sp.*	Taylor Co., Fla.	8/54	l, s, fl, r	–	0	0	0
5393	*Crotonopsis linearis*	Leon Co., Fla.	7/55	w	–	0	0	..
5608	*Cryptantha albida*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..
5511	*Ditaxis blodgettii*	Monroe Co., Fla.	8/55	l, s, r	–	0	0	..
5497	*Euphorbia buxifolia*	Monroe Co., Fla.	8/55	w	–	+	0	..
5509	*Euphorbia heterophylla*	Monroe Co., Fla.	8/55	w	–	0	0	..
5523	*Euphorbia sp.*	Lee Co., Fla.	8/55	l, s	–	+	0	..
5146	*Euphorbia sp.*	Baltimore Co., Md.	9/54	l, s, r, fl	–	0	0	..
5697	*Jatropha spathulata*	Val Verde Co., Tex.	9/55	l, tw	–	0	0	..
6291	*Jatropha spathulata*	Candellaria, Tex.	4/57	s	–	0	0	..
5087	*Ricinus communis*	Pinellas Co., Fla.	8/54	l, s	–	0	0	..
5098	*Stillingia spathulata*	Orange Co., Fla.	8/54	l, s, r, fr	–	0	0	..
5749	*Stillingia sp.*	Ward Co., Tex.	9/55	l, s, fr	–	+	0	..
5644	*Tragia Neo Mexicana*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	0	..
		FAGACEAE						
5050	*Castanea alnifolia*	Levy Co., Fla.	8/54	l, s	–	0	0	0
5008	*Fagus grandifolia*	Winsted, Conn.	8/54	fr	–	+	0	+++
6323	*Quercus albocincta*	Sonora, Mexico	5/57	b	–	+++	0	..
5745	*Quercus havardii*	Ward Co., Tex.	9/55	l, tw	–	0	0	..
5241	*Quercus imbricaria*	Baltimore Co., Md.	10/54	l, s	–	0	0	..
5442	*Quercus myrtifolia*	Highlands Co., Fla.	8/55	l, tw	–	0	0	..
5248	*Quercus Virginiana* (*Q. minima*)	Calhoun Co., Fla.	10/54	l, s	–	0	0	..
		FOUQUIERIACEAE						
5557	*Fouquieria splendens*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..
6397	*Idria columnaris*	Rosario, Baja Calif., Mexico	7/57	b	+	0	0	..
		GNETACEAE						
5605	*Ephedra Nevadensis* var. *aspera* (*E. aspera*)	Brewster Co., Tex.	8/55	l, s	–	0	0	..
		GOODENIACEAE						
5075	*Scaevola plumieri*	Pinellas Co., Fla.	8/54	l, s, fr	–	0	0	++

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		GRAMINEAE						
5153	*Andropogon glomeratus*	Windsor, N. C.	9/54	l, r	−	0	0	..
5077	*Andropogon littoralis*	Pinellas Co., Fla.	8/54	l, s, r, fl	−	+	0	0
5254	*Andropogon sp.*	Liberty Co., Fla.	10/54	l, r	−	0	0	..
5255	*Andropogon sp.*	Liberty Co., Fla.	10/54	l, r	−	++	0	..
5220	*Anthaenantia villosa*	Wakulla Co., Fla.	10/54	l, r, fr	−	0	0	..
5976	*Arrhenatherum elatius*	Anne Arundel Co., Md.	6/56	w	−	0	0	..
6062	*Arundo donax*	Chico, Calif.	8/56	s	−	0	0	0
6063	*Arundo donax*	Chico, Calif.	8/56	l, s	−	0	0	0
6064	*Arundo plinii*	Chico, Calif.	8/56	l, s	−	0	0	0
5958	*Avena fatua*	King George Co., Va.	5/56	l, r	−	0	0	..
5138	*Bambusa dissimulator*	C. G. F.	9/54	l, s	−	0	0	..
5141	*Bambusa longispiculata*	C. G. F.	9/54	l, s	−	0	0	..
5139	*Bambusa malingensis*	C. G. F.	9/54	l, s	−	0	0	..
5140	*Bambusa multiplex*	C. G. F.	9/54	l, s	−	0	0	..
5494	*Bambusa pervariabilis*	C. G. F.	8/55	l, s	−	0	0	..
5517	*Bambusa polymorpha*	C. G. F.	8/55	l, s	−	0	0	..
5142	*Bambusa textilis*	C. G. F.	9/54	l, s	−	0	0	..
5478	*Bambusa vulgaris*	C. G. F.	8/55	l, s	−	0	0	..
5183	*Cenchrus echinatus*	Leon Co., Fla.	9/54	l, r, fr	−	0	0	..
5078	*Chloris glauca*	Pinellas Co., Fla.	9/54	l, r	−	+	0	0
5944	*Dactylis glomerata*	Baltimore Co., Md.	5/56	l, r, fr	−	0	0	..
5952	*Danthonia spicata*	Charles Co., Md.	5/56	l, s, r	−	0	0	..
5954	*Deschampsia flexuosa*	Charles Co., Md.	5/56	l, s, r	−	0	0	..
5219	*Digitaria villosa*	Wakulla Co., Fla.	10/54	l, s, r, fl	−	0	0	..
5230	*Digitaria villosa*	Jefferson Co., Fla.	10/54	l, r, fr	−	0	0	..
5061	*Distichlis spicata*	Levy Co., Fla.	8/54	l, r	−	0	0	0
5104	*Distichlis spicata*	Queen Annes Co., Md.	8/54	l, s, r, fl	−	0	0	..
5403	*Echinochloa colonum*	Leon Co., Fla.	7/55	l, s	−	0	0	..
5058	*Echinochloa crusgalli*	Levy Co., Fla.	8/54	l, s, fl	−	0	0	0
5107	*Echinochloa crusgalli*	Sussex Co., Del.	8/54	l, r, fl	−	0	0	..
5128	*Elymus Virginicus*	York Co., Pa.	8/54	l, s, r, fl	−	0	0	..
5257	*Eragrostis elliottii*	Liberty Co., Fla.	10/54	l, r	−	0	0	..
5217	*Erianthus giganteus*	Wakulla Co., Fla.	10/54	l, r, fr	−	0	0	..
5150	*Erianthus sp.*	Petersburg, Va.	9/54	l, r, fr	−	0	0	..
5004	*Heteropogon melanocarpus*	Leon Co., Fla.	8/54	l, s, r	−	0	0	0
5847	*Holcus lanata*	Baltimore Co., Md.	5/56	l, s, r, fl	−	0	0	..
5983	*Hordeum vulgare*	Baltimore Co., Md.	5/56	w	−	0	0	..
5953	*Lolium multiflorum*	Charles Co., Md.	5/56	l, s, r	−	0	0	..
5252	*Manisuris sp.*	Calhoun Co., Fla.	10/54	l, r	−	0	0	..
5125	*Muhlenbergia cf. frondosa*	York Co., Pa.	8/54	l, r	−	0	0	..
5126	*Muhlenbergia sp.*	York Co., Pa.	8/54	l, fl	−	0	0	..
5127	*Muhlenbergia sp.*	York Co., Pa.	8/54	l, r, fl	−	0	0	..
5391	*Panicum ashei*	Leon Co., Fla.	7/55	w	−	0	0	..
5108	*Panicum sp.*	Sussex Co., Del.	8/54	l, r, fr	−	0	0	..
6011	*Parapholis incurva* (*Pholiurus incurvus*)	Va.	7/56	w	−	0	0	..

5115	*Paspalum*	Sussex Co., Del.	8/54	l, r, fr	–	0	0	..
5975	*Phleum pratense*	Anne Arundel Co., Md.	6/56	w	–	0	0	..
6120	*Phyllostachys bambusoides*	Chico, Calif.	10/56	l, tw, wd	–	0	0	0
5949	*Poa pratensis*	Baltimore Co., Md.	5/56	l, s, r, fl	–	0	0	..
5231	*Sacciolepis striata*	Jefferson Co., Fla.	10/54	l, r, fr	–	0	0	..
6040	*Secale cereale*	Dundee, N. Y.	7/56	l, s, r	–	+	0	..
5149	*Setaria cf. corrugata*	Caroline Co., Va.	9/54	l, r, fl	–	0	0	..
5184	*Setaria lutescens*	Leon Co., Fla.	9/54	l, r, fr	–	0	0	..
5216	*Sorghastrum secundum*	Wakulla Co., Fla.	10/54	l, fl	–	0	0	..
5130	*Sorghum vulgare*	York Co., Pa.	8/54	l, s, r, fr	–	0	0	..
6126	*Thysanolaena maxima*	C. G. F.	10/56	l, s, fl	–	0	0	0
5090	*Rhynchelytrum roseum* (*Tricholaena rosea*)	Polk Co., Fla.	8/54	l, r, fl	–	0	0	..
5984	*Triticum aestivum*	Baltimore Co., Md.	5/56	w	–	0	0	..
6018	*Zea mays*	Md.	7/56	l, s	–	0	0	..
		GUTTIFERAE						
6080A	*Garcinia spicata*	C. G. F.	9/54	l	–	+++	0	0
6080B	*Garcinia spicata*	C. G. F.	9/54	tw	–	+++	0	0
6080C	*Garcinia spicata*	C. G. F.	9/54	fr	–	+	0	0
6089A	*Garcinia spicata*	C. G. F.	9/56	l	–	+++	0	0
6089B	*Garcinia spicata*	C. G. F.	9/56	tw	–	+++	0	0
6089C	*Garcinia spicata*	C. G. F	9/56	fr	–	+	0	0
5375	*Hypericum sphaerocarpum* (*H. cistifolium*)	Gadsden Co., Fla.	7/55	l, tw	–	0	0	..
5864	*Vismia sp.*	Costa Rica	4/56	l, s	–	0	0	..
		HYDROPHYLLACEAE						
5682	*Nama havardii*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
6423	*Phacelia sp.*	Baja Calif., Mexico	8/57	fl	–	++	0	..
		JUGLANDACEAE						
5232	*Carya aquatica*	Jefferson Co., Fla.	10/54	l, s	–	0	0	..
5440A	*Carya Floridana*	Highlands Co., Fla.	8/55	l, tw	–	+	0	..
5440B	*Carya Floridana*	Highlands Co., Fla.	8/55	fr	–	0	0	..
5535	*Juglans microcarpa* (*J. rupestris*)	Jeff Davis Co., Tex.	8/55	fr	–	0	0	..
5581	*Juglans microcarpa* (*J. rupestris*)	Highlands Co., Fla.	8/55	l, s	–	0	0	..
		LABIATAE						
6162	*Coleus* (cult.)	Baltimore, Md.	11/56	l, s, r	–	0	0	0
5265	*Dicerandra linearifolia*	Leon Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5171	*Lepechinia confusa*	Glenn Dale, Md.	9/54	l, s	–	0	0	..
6424	*Salvia mellifera*	Baja Calif., Mexico	8/57	fl	–	0	+	..
5247	*Lycopus sp.*	Calhoun Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5556	*Marrubium vulgare*	Jeff Davis Co., Tex.	8/55	l, s, r, fr	–	0	+	..
5518	*Melaleuca leucodendron*	Lee Co., Fla.	8/55	l, tw, fr	–	+	0	..
5029	*Mentha Canadensis* (*M. arvensis* var. *villosa*)	Berkshire Co., Mass.	8/54	l, s, fl, r	–	0	0	0
5600	*Monarda cf. punctata*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0	..
6070	*Origanum vulgare*	Millerton, N. Y.	9/56	l, s, r, fl	–	0	0	0
5653	*Tetraclea coulteri*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	+	..
5010	*Trichostema dichotomum*	Torrington, Conn.	8/54	l, s, r	–	0	++	0
		LAURACEAE						
3789	*Cinnamomum camphora*	McIntosh Co., Ga.	4/54	l	..	..	+	..
5445	*Persea Americana*	Palm Beach Co., Fla.	8/55	l, tw, fr	–	0	0	..

Accession No.	Species	Collection Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		LAURACEAE						
5441	*Persea humilis*	Highlands Co., Fla.	8/55	l, tw	−	+	0	..
5861	*Phoebe Mexicana*	Costa Rica	4/56	l, s, b	−	0	0	..
5458A	*Umbellularia Californica*	Chico, Calif.	8/55	fr	−	0	+	0
5458B	*Umbellularia Californica*	Chico, Calif.	8/55	l, r	−	0	0	0
		LEGUMINOSAE						
5711	*Acacia berlandieri*	Val Verde Co., Tex.	9/55	l, s	−	0	+	..
6203	*Acacia choriophylla*	C. G. F.	2/57	l, tw	−	0	0	0
6087A	*Acacia confusa*	C. G. F.	9/56	l	−	+	0	+
6087B	*Acacia confusa*	C. G. F.	9/56	tw	−	0	0	0
6087C	*Acacia confusa*	C. G. F.	9/56	fr	−	0	0	0
5579	*Acacia cuspidata*	Brewster Co., Tex.	8/55	l, s, fl	−	0	0	..
6204	*Acacia koa*	C. G. F.	2/57	l, tw	−	0	0	0
6313	*Acacia occidentalis*	Sonora, Mexico	5/57	b	+	0	0	..
6213A	*Acacia pallens*	C. G. F.	7/57	l	−	0	0	+
6213B	*Acacia pallens*	C. G. F.	7/57	b	−	0	0	+
6213C	*Acacia pallens*	C. G. F.	7/57	r	−	0	0	+
6206	*Acacia pallens*	C. G. F.	2/57	l, tw	−	0	0	0
6152A	*Acacia sowdeni*	Chico, Calif.	11/56	l, s	−	0	0	+
6152B	*Acacia sowdeni*	Chico, Calif.	11/56	tw	−	0	0	0
5092	*Aeschynomene Americana*	Polk Co., Fla.	8/54	l, s	−	0	0	..
6026-O	*Albizzia julibrissin*	Charleston, S. C.	10/57	l, tw, b	−	0	0	..
5480	*Albizzia lebbeck*	Monroe Co., Fla.	8/55	l, tw	−	0	0	..
6053A	*Albizzia polyphylla*	C. G. F.	8/56	l	−	0	+++	0
6053B	*Albizzia polyphylla*	C. G. F.	8/56	tw	−	0	+	0
6053C	*Albizzia polyphylla*	C. G. F.	8/56	r	−	0	0	0
5228	*Alysicarpus vaginalis*	Jefferson Co., Fla.	10/54	l, s, r, fr	−	0	0	..
5089	*Amorpha cf. dewinkeleri*	Polk Co., Fla.	8/54	l, s	−	+	0	..
5625	*Astragalus earlei*	Jeff Davis Co., Tex.	8/55	l, s, fl	−	0	0	..
5986	*Astragalus earlei*	Brewster Co., Tex.	6/56	l, s, r, fl	−	0	0	0
6289	*Astragalus earlei*	Alpine, Tex.	4/57	l, s, fl	−	0	0	..
5960-O	*Astragalus leucophyllus*	Alameda Co., Calif.	6/57	w	−	0	0	..
5144	*Bauhinia aculeata*	C. G. F.	9/54	l, s	−	0	0	..
5143	*Bauhinia acuminata*	C. G. F.	9/54	l, s	−	0	0	..
5597	*Bauhinia forficata*	C. G. F.	8/55	l, s	−	0	0	..
5191	*Bauhinia hupehana*	C. G. F.	9/54	l, s	−	0	0	..
5192	*Bauhinia millicella*	C. G. F.	9/54	l, s, fr	−	0	0	..
5193	*Bauhinia pauletia*	C. G. F.	9/54	l, s	−	0	0	..
5188	*Bauhinia polycarpa*	C. G. F.	9/54	l, s	−	0	0	..
5195	*Bauhinia reticulata*	C. G. F.	9/54	l, s	−	0	0	..
5596	*Bauhinia retusa*	C. G. F.	8/55	l, s	−	0	0	..
6165A	*Bauhinia vahlii*	C. G. F.	11/56	l	−	+++	0	+
6165B	*Bauhinia vahlii*	C. G. F.	11/56	tw	−	0	0	+
5194	*Bauhinia variegata*	C. G. D.	9/54	l, s	−	0	0	..
5196	*Bauhinia variegata*	C. G. F.	9/54	l, s	−	0	0	..
3006	*Caesalpinia vesicaria*	C. G. F.	8/52	l	−	..	+	..

6415	*Calliandra tonduzii*	Chiapas, Mexico	8/57	l, s	–	0	0		..	
5059	*Cassia aspera*	Levy Co., Fla.	8/54	l, s, r, fl	–	0	0		+	
5640	*Cassia bauhinioides*	Brewster Co., Tex.	8/55	l, s, r	–	0	0		..	
5639	*Cassia durangensis*	Brewster Co., Tex.	8/55	l, s, r	–	0	0		..	
6066A	*Cassia excelsa*	C. G. F.	8/56	l	–	0	++		0	
6066B	*Cassia excelsa*	C. G. F.	8/56	tw	–	0	0		0	
6066C	*Cassia excelsa*	C. G. F.	8/56	fl	–	0	+++		0	
5539	*Cassia lindheimeriana*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0		..	
5638	*Cassia lindheimeriana*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0		..	
5560	*Cassia roemeriana*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0		..	
6075A	*Cassia spectabilis*	C. G. F.	9/56	l	–	0	0		0	
6075B	*Cassia spectabilis*	C. G. F.	9/56	tw	–	0	0		0	
6076A	*Cassia spectabilis*	C. G. F.	9/56	l	–	0	++		0	
6076B	*Cassia spectabilis*	C. G. F.	9/56	tw	–	0	0		0	
5522	*Cassia sp.*	Collier Co., Fla.	8/55	l, s	–	0	0		..	
6078A	*Ceratonia siliqua*	C. G. F.	9/56	l, s	–	+++	0		+++	
6078B	*Ceratonia siliqua*	C. G. F.	9/56	tw	–	0	0		++	
6078C	*Ceratonia siliqua*	C. G. F.	9/56	fr	–	0	0		0	
5102	*Chapmannia Floridana*	Lake Co., Fla.	8/54	l, s, r	–	0	0		..	
5432	*Chapmannia Floridana*	Lake Co., Fla.	8/55	w	–	0	0		..	
5267	*Crotalaria intermedia*	Leon Co., Fla.	10/54	l, s, fr	–	0	0		..	
5165	*Crotalaria spectabilis*	Leon Co., Fla.	9/54	l, s, fr	–	0	0		..	
5259	*Crotalaria usaramoensis*	Liberty Co., Fla.	10/54	l, s, r, fr	–	0	0		..	
5459	*Dalbergia brownei*	Broward Co., Fla.	8/55	l, tw	–	0	0		..	
5735	*Dalea alopecuroides*	Jeff Davis Co., Tex.	9/55	l, s, r	–	0	+		..	
5709	*Dalea argyraea*	Val Verde Co., Tex.	9/55	l, s	–	0	0		..	
5565	*Dalea aurea*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0		..	
5601	*Dalea frutescens*	Jeff Davis Co., Tex.	8/55	l, s, r	–	0	0		..	
5589	*Dalea lachnostachys*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0		..	
5742	*Dalea terminalis*	Ward Co., Tex.	9/55	l, s, fl	–	0	+		..	
5422	*Daubentonia drummondii*	Jackson Co., Miss.	8/55	l, s, fr	–	0	0		..	
5444	*Delonix regia*	Palm Beach Co., Fla.	8/55	l, tw	–	0	0		..	
5487	*Delonix regia*	Monroe Co., Fla.	8/55	fr	–	0	0		..	
5583	*Desmanthus cooleyi*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0		..	
5056	*Desmodium canum*	Levy Co., Fla.	8/54	l, s, r, fl	–	0	0		0	
6293	*Enterolobium cyclocarpum*	Cuyotenango, Guatemala	4/57	sd	–	0	0		..	
5269	*Erythrina herbacea*	Leon Co., Fla.	10/54	l, s	–	0	+		..	
5371A	*Erythrina herbacea*	Leon Co., Fla.	7/55	l, s	–	0	+		..	
5371B	*Erythrina herbacea*	Leon Co., Fla	7/55	r	–	0	++		..	
5706	*Eysenhardtia angustifolia*	Brewster Co., Tex.	8/56	l, tw, fr	–	0	0		..	
6045	*Hedysarum sp.*	Alberta, Canada	8/56	l, s	–	0	0		..	
5592	*Hoffmanseggia drepanocarpa*	Brewster Co., Tex.	8/55	l, s, fl, fr	–	0	0		..	
5438	*Indigofera hirsuta*	Highlands Co., Fla.	8/55	l, s, r, fl	–	0	0		..	
5708	*Indigofera lindheimeriana*	Val Verde Co., Tex.	9/55	l, s	–	0	0		..	
6407	*Inga laurina*	Chiapas, Mexico	8/57	l, s	–	0	0		..	
5878	*Inga sp.*	Costa Rica	4/56	l, s, b	–	0	0		..	
5731	*Krameria grayi*	Ward Co., Tex.	9/55	w	–	0	0		..	
5483	*Leucaena leucocephala*	Monroe Co., Fla.	8/55	l, tw	–	0	0		..	
5568	*Leucaena retusa*	Jeff Davis Co., Tex.	8/55	l, tw, fr	–	+	0		..	
5420	*Lupinus westiana*	Oskaloosa Co., Fla.	8/55	w	–	0	++		..	

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		LEGUMINOSAE						
5481	*Lysiloma Bahamensis*	Monroe Co., Fla.	8/55	l, tw	–	0	0	..
6320	*Lysiloma microphylla* (*L. watsonii*)	Sonora, Mexico	5/57	b	–	0	0	..
5870-O	*Melilotus alba*	Montgomery Co., Pa.	7/56	l	–	0	0	..
5881-O	*Melilotus officinalis*	Lester, Pa.	8/56	l, fl	–	0	0	..
5622	*Mimosa flexuosa*	Jeff Davis Co., Tex.	8/55	l, tw	–	0	0	..
5460	*Neptunia Floridana*	Broward Co., Fla.	8/55	w	–	0	0	..
6084	*Oxytropis monticola*	Alberta, Canada	9/56	l, s, r, fl	–	0	0	0
6159A	*Peltophorum Africanum*	C. G. F.	11/56	l	–	++	0	+++
6159B	*Peltophorum Africanum*	C. G. F.	11/56	tw	–	0	0	++
6159C	*Peltophorum Africanum*	C. G. F.	11/56	fr	–	0	0	0
5047	*Petalostemon carneus*	Taylor Co., Fla.	8/54	l, s, fl, r	–	0	0	+
5529	*Petalostemon carneus*	Pinellas Co., Fla.	8/55	l, s, r, fl	–	0	0	..
5546	*Petalostemon oligophyllus*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5942-O	*Piscidia erythrina*	Clarendon, Jamaica, B. W. I.	5/57	b	–	0	..	0
5475	*Piscidia piscipula*	Monroe Co., Fla.	8/55	l, tw, fr	–	0	0	..
6065	*Pithecellobium flexicaule*	C. G. F.	8/56	l, tw	–	0	0	0
5488	*Pithecellobium keyense*	Monroe Co., Fla.	8/55	l, tw	–	0	0	..
6322	*Pithecellobium Mexicanum*	Sonora, Mexico	5/57	b	–	0	0	..
6419	*Pithecellobium pachypus*	Chiapas, Mexico	8/57	l, s, fr	–	0	0	..
6420	*Pithecellobium undulatum*	Sonora, Mexico	8/57	sd	–	0	0	..
5479	*Pithecellobium unguis-cati*	Monroe Co., Fla.	8/55	l, tw	–	0	0	..
5572	*Prosopis juliflora*	Brewster Co., Tex.	8/55	fr	–	0	0	..
5573	*Prosopis juliflora*	Brewster Co., Tex.	8/55	l, tw	–	0	++	..
6052A	*Pterogyne nitens*	C. G. F.	8/56	l	–	+	++	0
6052B	*Pterogyne nitens*	C. G. F.	8/56	l, s	–	+	++	0
6052C	*Pterogyne nitens*	C. G. F.	8/56	tw	–	0	0	+
5096	*Rhynchosia cinerea*	Orange Co., Fla.	8/54	l, s	–	+	0	..
5563	*Rhynchosia Texana*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..
6054A	*Samanea saman*	C. G. F.	8/56	l	–	0	++	0
6054B	*Samanea saman*	C. G. F.	8/56	tw	–	0	0	0
6160A	*Schotia Transvaalensis*	C. G. F.	11/56	l	–	++	0	++
6160B	*Schotia Transvaalensis*	C. G. F.	11/56	tw	–	0	0	++
5545	*Sophora secundiflora*	Jeff Davis Co., Tex.	8/55	l, tw	–	0	++	..
5713A	*Sophora secundiflora*	Val Verde Co., Tex.	9/55	sd	–	0	0	..
5713B	*Sophora secundiflora*	Val Verde Co., Tex.	9/55	fr	–	0	++	..
5088	*Sophora tomentosa*	Pinellas Co., Fla.	8/54	l, s, fr	–	0	++	..
5880-O	*Strophostyles helvola*	Philadelphia, Pa.	8/56	l	–	0	0	..
6412	*Sweetia Panamensis*	Chiapas, Mexico	8/57	l	–	0	0	..
5099	*Tamarindus indica*	Lake Co., Fla.	8/54	l, s, fr	–	0	0	..
6205A	*Trachylobium hornemannianum*	C. G. F.	8/54	l, s	–	0	+	0
6205B	*Trachylobium hornemannianum*	C. G. F.	8/54	tw	–	0	++	++
5956	*Vicia sp.* (cult.)	Charles Co., Md.	5/56	l, s, fl	–	0	0	..
5456	*Vigna marina*	Broward Co., Fla.	8/55	w	–	0	0	..
		LILIACEAE						
5204	*Allium cernuum*	Allegany Co., Md.	9/54	l, r, fl	–	0	0	..

5895-O	*Allium cernuum*	Perry Co., Pa.	8/56	l, fl, r	–	0	0	..	
5011	*Allium tricoccum*	Colebrook, Conn.	8/54	t, r	–	0	+	0	
6021-O	*Aloe vulgaris*	Kingston, Jamaica, B. W. I.	10/57	l	–	0	..	..	
5023	*Clintonia borealis*	Sandisfield, Mass.	8/54	l, s	–	0	0	0	
5864	*Eremurus sp.*	Kerman, Iran	5/55	l	–	0	..	..	
5365	*Eremurus sp.*	Kerman, Iran	5/55	r, crown	–	..	..	..	
5366	*Eremurus sp.*	Kerman, Iran	5/55		–	..	..	..	
6318	*Heliocarpus*	Sonora, Mexico	5/57	s	–	0	0	..	
5902-O	*Hemerocallis sp.*	Montgomery Co., Pa.	8/56	t, r	–	0	0	..	
6030-O	*Lilium auratum* var. *platyphyllum*	Gresham, Oreg.	10/57	bu	–	0	0	0	
6031-O	*Lilium Formosanum*	Gresham, Oreg.	10/57	bu	–	0	0	0	
6032-O	*Lilium hansonii*	Gresham, Oreg.	10/57	bu	–	0	0	0	
6033-O	*Lilium henryi*	Gresham, Oreg.	10/57	bu	–	0	0	0	
6034-O	*Lilium Japonicum*	Gresham, Oreg.	10/57	bu	–	0	0	0	
6035-O	*Lilium longiflorum*	Gresham, Oreg.	10/57	bu, r	–	0	0	0	
6036-O	*Lilium martagon* var. *album*	Gresham, Oreg.	10/57	bu, r	–	0	0	0	
6037-O	*Lilium Nepalense*	Gresham, Oreg.	10/57	bu	+	0	0	0	
6041-O	*Lilium pardalinum* var. *giganteum*	Gresham, Oreg.	10/57	bu, r	–	0	0	0	
6038-O	*Lilium pumilum*	Gresham, Oreg.	10/57	bu, r	–	0	0	0	
6039-O	*Lilium regale*	Gresham, Oreg.	10/57	bu, r	–	0	0	0	
6040-O	*Lilium speciosum*	Gresham, Oreg.	10/57	bu, r	–	0	0	0	
5986-O	*Lilium tigrinum*	Philadelphia, Pa.	7/57	l, s	–	0	0	..	
6003	*Maianthemum Canadense*	Winsted, Conn.	6/56	l, s, r	–	0	0	..	
6290	*Nolina Texana*	Alpine, Tex.	4/57	l, s, fl	–	0	0	..	
6388	*Sansevieria angustiflora*	Belle Glade, Fla.	7/57	l	+	0	0	0	
6395	*Sansevieria angustiflora*	Belle Glade, Fla.	7/57	rh	–	0	0	0	
6394	*Sansevieria desertii*	Belle Glade, Fla.	7/57	l	–	0	0	0	
6390	*Sansevieria trifasciata*	Belle Glade, Fla.	7/57	l	–	0	0	+	
6392	*Sansevieria trifasciata*	Belle Glade, Fla.	7/57	l	–	0	0	0	
6396	*Sansevieria trifasciata*	Belle Glade, Fla.	7/57	rh	–	0	0	0	
5009	*Smilax herbacea*	New Boston, Mass.	8/54	l, s, fr	–	0	0	0	
6001	*Streptopus roseus*	Winsted, Conn.	6/56	l, s, r	–	0	0	..	
6019	*Tulipa sp.*	Baltimore Co., Md.	7/56	l, s, fr, bu	–	0	0	..	
6020	*Uvularia perfoliata*	Queen Ann Co., Md.	7/56	l, s	–	0	0	..	
6081	*Xerophyllum tenax*	Alberta, Canada	8/56	l	–	0	0	..	
6047	*Zigadenus elegans*	Alberta, Canada	7/56	l, s	–	0	0	..	
		LOASACEAE							
5555	*Cevallia sinuata*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..	
5754	*Mentzelia sp.*	Ward Co., Tex.	9/55	l, s, fl	–	+	0	..	
		LOGANIACEAE							
5580	*Buddleia scordioides*	Jeff Davis Co., Tex.	8/55	l, tw, fl	–	0	0	..	
5412	*Spigelia Marilandica*	Jefferson Co., Fla.	7/55	w	–	0	0	..	
		LORANTHACEAE							
5562	*Phoradendron engelmannii*	Jeff Davis Co., Tex.	8/55	l, s, tw, fr	–	0	0	..	
		LYCOPIDIACEAE							
5012	*Lycopodium annotinum*	Colebrook, Conn.	8/54	fd	–	0	++	0	
5016	*Lycopodium clavatum*	Sheffield, Mass.	8/54	fd	–	0	++	0	
5021	*Lycopodium obscurum*	Sandisfield, Mass.	8/54	fd, r	–	0	++	0	

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		LYTHRACEAE						
5063	*Ammannia coccinea*	Levy Co., Fla.	8/54	l, s, fl, r	–	0	0	+++
5198	*Cuphea petiolata*	Allegany Co., Md.	9/54	l, s, r, fl	–	0	0	..
5048	*Lythrum lanceolatum*	Taylor Co., Fla.	8/54	l, s, r	–	0	+	++
5006	*Lythrum salicaria*	Ladentown, N. Y.	8/54	l, s, fl	–	0	0	+
5154	*Rotala ramosior*	Windsor, N. C.	9/54	l, s, r, fr	–	0	0	..
		MAGNOLIACEAE						
5378	*Illicium Floridanum*	Gadsden Co., Fla.	7/55	l, tw	–	0	0	..
5419	*Magnolia grandiflora*	Calhoun Co., Fla.	8/55	fr	–	0	0	..
5900-O	*Magnolia macrophylla*	Philadelphia, Pa.	8/56	l, fr	–	0	0	..
5901-O	*Magnolia stellata*	Philadelphia, Pa.	8/56	l, s	–	0	..	..
5898-O	*Magnolia Virginiana*	Burlington Co., N. J.	8/56	l	–	0	0	..
		MALPIGHIACEAE						
6161A	*Byrsonima crassifolia*	C. G. F.	11/56	l	–	+	0	++
6161B	*Byrsonima crassifolia*	C. G. F.	11/56	tw	–	0	0	+++
5502	*Byrsonima lucida*	Monroe Co., Fla.	8/55	l, tw	–	0	0	..
5652	*Janusia gracilis*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
		MALVACEAE						
5667	*Abutilon incanum*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
6312	*Abutilon sonorae*	Sonora, Mexico	5/57	r	–	0	0	..
6311	*Abutilon sonorae*	Sonora, Mexico	5/57	l, s	–	0	+	..
6042	*Althaea officinalis*	Monroe Co., N. Y.	7/56	l, s, fl	–	0	0	..
6007	*Althaea rosea*	Baltimore Co., Md.	7/56	l, s, sd	–	0	0	..
5473	*Gayoides crispum*	Monroe Co., Fla.	8/55	w	–	0	..	..
5632	*Hibiscus denudatus*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..
5080	*Hibiscus rosa-sinensis*	Pinellas Co., Fla.	8/54	l, s	–	0	0	0
5448	*Kosteletzkya altheaefolia*	Palm Beach Co., Fla.	8/55	w	–	0	0	..
5389	*Sida carpinifolia*	Leon Co., Fla.	7/55	w	–	0	0	..
5550	*Sphaeralcea angustifolia* var. *cuspidata*	Jeff Davis Co., Tex.	8/55	l, s, tw, fl	–	0	0	..
5612	*Sphaeralcea sp.*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..
5083	*Urena lobata*	Pinellas Co., Fla.	8/54	l, s	–	0	0	0
		MELIACEAE						
5135	*Azadirachta indica*	C. G. F.	9/54	l, s	–	0	0	..
6416	*Guarea trompillo*	Chiapas, Mexico	8/57	l, s	–	0	0	..
5755	*Melia azedarach*	Pecos Co., Tex.	9/55	l, s	–	0	0	..
5091	*Thalia geniculata*	Polk Co., Fla.	8/54	l, s	–	0	0	..
		MENISPERMACEAE						
5413	*Calycocarpum lyonii*	Jefferson Co., Fla.	7/55	w	–	0	0	..
		MORACEAE						
5450	*Ficus aurea*	Broward Co., Fla.	8/55	l, tw	–	0	0	..
5064	*Ficus carica*	Levy Co., Fla.	8/54	l, s	–	0	0	0
6316	*Ficus cotinifolia*	Sonora, Mexico	5/57	l	–	0	0	

5464	*Ficus laevigata* (*F. brevifolia*)	Dade Co., Fla.	8/55	l, tw	–	0	0	..	
5887-O	*Maclura pomifera*	Montgomery Co., Pa.	8/56	l	–	0	0	..	
6157	*Morus alba*	Chico, Calif.	11/56	l, tw	–	0	0	0	
5918-O	*Morus rubra*	Montgomery Co., Pa.	9/56	l	–	0	0	..	
		MYRICACEAE							
5423	*Myrica inodora*	Jackson Co., Miss.	8/55	l, tw	–	++	0	..	
		MYRSINACEAE							
5065	*Rapanea Guianensis*	Levy Co., Fla.	8/54	l, s	–	0	0	++	
5467	*Rapanea Guianensis*	Dade Co., Fla.	8/55	l, tw	–	0	0	..	
5860	*Rapanea sp.*	Atenas, Costa Rica	4/56	l, s, b	–	0	0	0	
		MYRTACEAE							
5492	*Eugenia anthera*	Monroe Co., Fla.	8/55	l, tw	–	+	0	..	
6170A	*Eugenia atropunctata*	C. G. F.	12/56	l	–	0	0	+++	
6170B	*Eugenia atropunctata*	C. G. F.	12/56	tw	–	0	0	++	
5489	*Eugenia myrtoides*	Monroe Co., Fla.	8/55	l, tw	–	0	0	..	
6122	*Feijoa sellowiana*	Chico, Calif.	10/56	l, s, wd	–	0	0	0	
6410	*Pimenta dioica*	Chiapas, Mexico	8/57	l, s	–	0	0	..	
5081	*Psidium guajava*	Pinellas Co., Fla.	8/54	l, s, fr	–	0	0	+	
5132	*Tristania conferta*	C. G. F.	8/54	l	–	0	0	..	
5297	*Tristania conferta*	C. G. F.	11/54	l, s	–	0	0	..	
5298	*Tristania conferta*	C. G. F.	11/54	l, s	–	0	0	..	
		NYCTAGINACEAE							
5651	*Acleisanthes longiflora*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..	
5669	*Allionia incarnata*	Brewster Co., Fla.	8/55	l, s, fl	–	0	0	..	
5282	*Boerhaavia coccinea*	St. Johns Co., Fla.	11/54	l, s, r	–	0	0	..	
5410	*Boerhaavia erecta*	Leon Co., Fla.	7/55	w	–	0	0	..	
5717	*Boerhaavia sp.*	Ward Co., Tex.	9/55	l, s	–	0	0	..	
5648	*Boerhaavia sp.*	Brewster Co., Tex.	8/55	l, s	–	0	0	..	
5714	*Cyphomeris gypsophiloides*	Brewster Co., Tex.	9/55	l, s, fl	–	0	0	..	
5666	*Nyctaginia capitata*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	0	..	
5739	*Oxybaphus sp.*	Ward Co., Tex.	9/55	l, tw, fl	–	0	0	..	
5641	*Selinocarpus angustifolius*	Brewster Co., Tex.	8/55	l, s, r	–	0	0	..	
5642	*Selinocarpus parvifolius*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0	..	
5491	*Torrubia bracei*	Monroe Co., Fla.	8/55	l, tw	–	0	0	..	
		NYMPHAEACEAE							
5051	*Brasenia schreberi*	Levy Co., Fla.	8/54	l, s	–	0	0	+	
5105	*Nelumbo nucifera*	Talbot Co., Md.	8/54	l, s, fl, fr	–	0	+	..	
		NYSSACEAE							
5677	*Nyssa ogeche*	Gulf Co., Fla.	9/55	l, tw, fr	–	0	0	..	
		OLEACEAE							
6128A	*Chionanthus retusa*	Chico, Calif.	10/56	l	–	+	0	..	
6128B	*Chionanthus retusa*	Chico, Calif.	10/56	tw	–	0	0	0	
6163A	*Chionanthus Virginica*	Chico, Calif.	11/56	l	–	++	0	0	
6163B	*Chionanthus Virginica*	Chico, Calif.	11/56	tw	–	0	0	0	
5524	*Forestiera pinetorum*	Lee Co., Fla.	8/55	l, tw	–	0	++	..	
5439	*Jasminum multiflorum*	Highlands Co., Fla.	8/55	l, tw	–	0	0	..	

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5689	*Menodora longiflora*	Terrell Co., Tex.	9/55	l, s, r, fl	–	0	0	..
6116A	*Olea Europaea*	Chico, Calif.	10/56	l	–	++	0	+++
6116B	*Olea Europaea*	Chico, Calif.	10/56	tw	–	0	0	0
5031	*Syringa vulgaris*	New Boston, Mass.	8/54	l, s	–	0	0	0
		ONAGRACEAE						
5722	*Gaura engelmannii*	Ward Co., Tex.	9/55	l, s	–	0	0	..
5398	*Gaura parviflora*	Leon Co., Fla.	7/55	w	–	0	0	..
5085	*Jussiaea angustifolia*	Pinellas Co., Fla.	8/54	l, s	–	0	0	..
5155	*Ludwigia alata*	Windsor, Conn.	9/54	l, s, r, fl	–	0	0	..
5399	*Ludwigia arcuata*	Leon Co., Fla.	7/55	w	–	0	0	..
5274	*Ludwigia natans*	Wakulla Co., Fla.	10/54	l, s, r, fl	–	0	0	..
5226	*Ludwigia sphaerocarpa*	Leon Co., Fla.	10/54	l, s, fl	–	0	0	..
5602	*Oenothera greggii*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	0	0	..
5003	*Oenothera sp.*	Leon Co., Fla.	8/54	l, s, r	–	0	0	+++
5729	*Oenothera sp.*	Ward Co., Tex.	9/55	l, s, fl	–	0	0	..
		OPHIOGLOSSACEAE						
6036	*Botrychium Virginianum*	Washington Co., Md.	7/56	l, s	–	0	0	..
		OROBANCHACEAE						
6071	*Epifagus (Epiphegus) Virginiana*	Winsted, Conn.	9/56	l, s, r	–	0	0	++
		ORCHIDACEAE						
6033	*Cypripedium acaule*	Anne Arundel Co., Md.	7/56	l, s, r	–	0	0	..
6072	*Epipactis helleborine*	Southfield, Mass.	9/56	l, s, fl	–	0	0	0
		PALMAE						
6202	*Acrocomia sclerocarpa*	C. G. F.	2/57	l, tw, fr	–	0	0	0
5133	*Astrocaryum Mexicanum*	C. G. F.	9/54	l, s	–	0	0	..
5186	*Astrocaryum standleyanum*	C. G. F.	9/54	l, s	–	0	0	..
5134	*Attalea spectabilis*	C. G. F.	9/54	l, s	–	0	0	..
5136	*Bactris pavoniana*	C. G. F.	9/54	l, s, fr	–	0	0	..
5307	*Chamaedorea erumpens*	C. G. F.	11/54	l, s	+	0	0	0
6166A	*Copernicia cerifera*	C. G. F.	11/56	l	–	+	0	+
6166B	*Copernicia cerifera*	C. G. F.	11/56	tw	–	0	0	0
6125A	*Heterospathe elata*	C. G. F.	10/56	l	–	0	0	++
6125B	*Heterospathe elata*	C. G. F.	10/56	fl	–	0	0	0
6125C	*Heterospathe elata*	C. G. F.	10/56	tw	–	0	0	0
6125D	*Heterospathe elata*	C. G. F	10/56	fr	–	0	0	+++
5187	*Orbignya barbosiana*	C. G. F.	9/54	l, husk, fr	–	0	0	..
5197	*Polyandrococcus caudescens*	C. G. F.	9/54	l, s	–	0	0	..
5131	*Pseudophoenix vinifera*	C. G. F.	8/54	l, s	–	0	0	..
5363	*Pseudophoenix vinifera*	C. G. F.	5/55	fr, s	–	0	0	..
6154	*Pseudophoenix vinifera*	C. G. F.	11/56	fr	–	0	0	0
6200A	*Pseudophoenix vinifera*	C. G. F.	1/57	l	–	0	0	0
6200B	*Pseudophoenix vinifera*	C. G. F.	1/57	tw	–	0	0	+
6200C	*Pseudophoenix vinifera*	C. G. F.	1/57	fr	–	0	0	0

6200D	*Pseudophoenix vinifera*	C. G. F.	1/57	s	–	0	0	++	
5049	*Sabal palmetto*	Taylor Co., Fla.	8/54	s, fr	–	0	0	0	
5678	*Sabal palmetto*	Gulf Co., Fla.	9/55	fr	–	0	0	..	
6386	*Sabal sp.*	Belle Glade, Fla.	7/57	petiole	–	0	0	0	
6389	*Sabal sp.*	Belle Glade, Fla.	7/57	l	–	0	0	0	
		PAPAVERACEAE							
5549	*Argemone platycerus*	Brewster Co., Tex.	8/55	l, s, fl	–	0	+	..	
5946	*Dicentra Canadensis*	Harford, Md.	5/56	l, s, r, fr	–	0	++	..	
6422	*Dicentra ochroleuca*	Baja California, Mexico	8/57	fr	–	+	++	..	
6014A	*Macleaya cordata (Bocconia cordata)*	Virginia	7/56	l, s	–	0	+++	..	
6014B	*Macleaya cordata (Bocconia cordata)*	Virginia	7/56	r	–	0	+++	..	
5982	*Papaver dubium*	Baltimore Co., Md.	5/56	l, s, fl	–	0	0	..	
6010	*Sanguinaria Canadensis*	Talbot Co., Md.	7/56	l, s	–	0	+	..	
		PASSIFLORACEAE							
5451	*Passiflora pallida (P. suberosa)*	Broward Co., Fla.	8/55	l, tw	–	0	0	..	
		PHRYMACEAE							
5206	*Phryma leptostachya*	Barrett Co., Md.	9/54	l, s, r	–	0	0	..	
		PHYTOLACCACEAE							
5095	*Phytolacca rigida*	Polk Co., Fla.	8/54	l, s, fr	–	+	0	..	
		PINACEAE							
5759	*Cupressus Arizonica*	Brewster Co., Tex.	10/55	l, tw	–	0	0	..	
5584	*Juniperus deppeana (J. pachyphloea)*	Jeff Davis Co., Tex.	8/55	l, s, fr	–	0	0	..	
5540	*Juniperus monosperma*	Jeff Davis Co., Tex.	8/55	l, tw	–	0	0	..	
5027	*Larix laricina*	Berkshire Co., Mass.	8/54	l, s	–	0	0	+	
5421	*Pinus clausa*	Okaloosa Co., Fla.	8/55	l, tw	–	0	0	..	
5382	*Pinus glabra*	Liberty Co., Fla.	7/55	l, tw	–	0	0	..	
5148	*Pinus Virginiana*	Baltimore Co., Md.	9/54	l, s, fr	–	0	0	..	
		PITTOSPORACEAE							
6042-O	*Pittosporum tobira*	Charleston, S. C.	10/57	l	–	0	..	0	
		POLEMONEACEAE							
5430	*Gilia rubra*	Lake Co., Fla.	8/55	w	–	0	0	..	
5244	*Phlox subulata*	Baltimore Co., Md.	10/54	l, s, r	–	0	0	..	
		POLYGALACEAE							
5068	*Polygala rugelii*	Citrus Co., Fla.	8/54	l, s, r, fl	–	+	+	0	
		POLYGONACEAE							
5512	*Antigonon leptopus*	Dade Co., Fla.	8/55	l, s	–	0	0	..	
5073	*Coccoloba uvifera*	Pinellas Co., Fla.	8/54	l, s	–	++	0	++	
5082	*Coccoloba uvifera*	Pinellas Co., Fla.	8/54	l, s	–	0	0	++	
5756	*Eriogonum annuum*	Ward Co., Tex.	9/55	l, s, r, fl	–	0	0	..	
5434A	*Eriogonum Floridanum*	Polk Co., Fla.	8/55	l, s, fl	–	0	0	..	
5434B	*Eriogonum Floridanum*	Polk Co., Fla.	8/55	r	–	0	++	..	
5757	*Eriogonum wrightii*	Brewster Co., Tex.	10/55	l, s, fl	–	0	0	..	
5109	*Polygonum hydropiper*	Sussex Co., Del.	8/54	l, s, r, fl	–	0	0	..	
5145	*Polygonum lapathifolium*	Baltimore Co., Md.	9/54	l, s, r, fl, fr	–	0	0	..	
5122	*Polygonum orientale*	York Co., Pa.	8/54	l, s, r	–	0	0	..	

Accession No.	Species	Collection Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
5147	*Polygonum persicaria*	Baltimore Co., Md.	9/54	l, s, r, fr	–	0	0	..
5043	*Polygonum punctatum*	Taylor Co., Fla.	8/54	l, s, fl	–	0	0	0
		POLYPODIACEAE						
5066	*Acrostichum danaeaefolium*	Levy Co., Fla.	8/54	fd	–	0	0	0
5015	*Athyrium filix-femina*	Colebrook, Conn.	8/54	fd, r	–	0	0	0
6038	*Cheilanthes vestita*	Washington Co., Md.	7/56	fd	–	+	0	..
5516	*Phlebodium aureum* (*Polypodium a.*)	Collier Co., Fla.	8/55	fd, r	–	0	0	..
5013	*Pteretis Pensylvanica*	Colebrook, Conn.	8/54	fd, r	–	0	0	0
5462	*Pteris Bahamensis*	Dade Co., Fla.	8/55	fd	–	0	0	..
5461	*Pteris vittata*	Dade Co., Fla.	8/55	fd	–	0	0	..
6037	*Woodsia obtusa*	Washington Co., Md.	7/56	fd, r	–	0	..	..
5974	*Woodwardia areolata* (*Lorinseria areolata*)	Anne Arundel Co., Md.	6/56	r	–	0	0	..
		POLYTRICHACEAE						
5407	*Atrichum sp.*	New Orleans, La.	7/55	w	–	0	0	..
		PONTEDERIACEAE						
5052	*Eichhornia crassipes*	Levy Co., Fla.	8/54	l, s, fl, r	–	0	0	0
5447	*Piaropus paniculatus*	Palm Beach Co., Fla.	8/55	w	–	0	0	..
		PORTULACACEAE						
5676	*Portulaca oleracea*	Brewster Co., Tex.	8/55	w	–	0	0	..
5530	*Portulaca pilosa*	Pinellas Co., Fla.	8/55	l, s, r, fl	–	0	0	..
5675	*Talinum aurantiacum*	Brewster Co., Tex.	8/55	l, s, fr	–	0	0	..
		POTAMOGETONACEAE						
6030	*Potamogeton pectinatus*	Washington, D. C.	7/56	l	–	..	..	..
6032	*Potamogeton ramosus*	Washington, D. C.	7/56	l, s	–	0	0	..
		PRIMULACEAE						
5979	*Anagallis arvensis*	Frederick Co., Md.	6/56	l, s, r, fl, fr	–	0	0	..
5024	*Lysimachia ciliata*	Sandisfield, Mass.	8/54	l, s, fr, r	–	0	0	0
6069	*Samolus parviflorus*	Harford Co., Md.	8/56	l, s, r, fl	–	0	0	+
6000	*Trientalis borealis*	Winsted, Conn.	6/56	l, s, r	–	0	0	..
		PROTEACEAE						
5521	*Grevillea robusta*	Lee Co., Fla.	8/55	l, tw	–	0	0	..
		PYROLACEAE						
5210	*Monotropa uniflora*	Baltimore Co., Md.	10/54	l, s, fl	–	0	0	..
		RANUNCULACEAE						
6006	*Actaea alba*	Winsted, Conn.	6/56	l, s, r, fr	–	0	0	..
6073	*Actaea rubra*	Southfield, Mass.	9/56	l, s, r	–	0	0	0
5955	*Aquilegia Canadensis*	Charles Co., Md.	5/56	l, s, r, fl	–	0	0	..
5931	*Caltha palustris*	Frederick Co., Md.	5/56	l, s, fl	–	0	0	..
5591	*Clematis drummondii* (male)	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..
5001	*Clematis Virginiana*	Leon Co., Fla.	8/54	l, s	–	0	0	0
5890-O	*Clamatis Virginiana*	Perry Co., Pa.	8/56	l	–	0	0	..

5924	*Delphinium tricorne*	Washington Co., Md.	5/56	l, s, fl	–	0	++	..
		RHAMNACEAE						
5433A	*Ceanothus microphyllus*	Polk Co., Fla.	8/55	l, s	–	++	++	..
5433B	*Ceanothus microphyllus*	Polk Co., Fla.	8/55	r	–	0	++	..
6319	*Karwinskia humboldtiana*	Sonora, Mexico	5/57	l	–	+++	0	..
5701	*Karwinskia humboldtiana*	Val Verde Co., Tex.	9/55	l, s, fr	–	0	0	..
6429	*Rhamnus crocea* var. *ilicifolia*	Baja California, Mexico	9/57	sd	–	++	0	..
6178A	*Rhamnus utilis*	Chico, Calif.	12/56	l, s	–	0	0	0
6178B	*Rhamnus utilis*	Chico, Calif.	12/56	wd	–	0	0	0
		RHIZOPHORACEAE						
5453A	*Rhizophora mangle*	Broward Co., Fla.	8/55	l, tw	–	+	0	..
5453B	*Rhizophora mangle*	Broward Co., Fla.	8/55	fr	–	0	0	..
6029-O	*Rhizophora mangle*	Port au Prince, Haiti	10/57	b	–	0	0	+++
		ROSACEAE						
5886-O	*Agrimonia parviflora*	Montgomery Co., Pa.	8/56	l, r	–	0	0	..
5899-O	*Amelanchier Canadensis*	Perry Co., Pa.	8/56	l	–	0	0	..
5455	*Chrysobalanus icaco*	Broward Co., Fla.	8/55	l, tw	–	0	0	..
5152	*Cotoneaster pyracantha*	Edenton, N. C.	9/54	l, s, r, fr	–	0	0	..
5208	*Crataegus crus-galli*	Garrett Co., Md.	9/54	l, s, fr	–	0	0	..
5207	*Crataegus punctata*	Garrett Co., Md.	9/54	l, s	–	0	0	..
5205	*Crataegus sp.*	Garrett Co., Md.	9/54	l, s, fl	–	0	0	..
5885-O	*Crataegus sp.*	Montgomery Co., Pa.	8/56	l, fr	–	0	0	..
6085	*Dryas drummondii*	Hosmer, B. C., Canada	7/56	l, s, r	–	0	0	++
5623	*Fallugia paradoxa*	Jeff Davis Co., Tex.	8/55	l, fl, tw	–	0	0	..
5981	*Fragaria Virginiana*	Frederick Co., Md.	6/56	l, s, r	–	0	0	..
6124A	*Licania rigida*	C. G. F.	10/56	l	–	+	0	++
6124B	*Licania rigida*	C. G. F.	10/56	tw	–	0	0	0
6167A	*Licania rigida*	C. G. F.	11/56	l	–	+	0	+++
6167B	*Licania rigida*	C. G. F.	11/56	tw	–	0	0	+
5026	*Potentilla fruticosa*	Berkshire Co., Mass.	8/54	l, s, fl, r	–	0	0	+++
5162	*Prunus Caroliniana*	Leon Co., Fla.	9/54	l, s, r, fl	–	0	0	..
5110	*Prunus persica*	Sussex Co., Del.	8/54	l, s	–	0	0	..
5620	*Prunus virens*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..
5876-O	*Rosa sp.*	Ocean City, N. J.	8/56	l, fr	–	0	0	..
5884-O	*Spiraea latifolia*	Bucks Co., Pa.	8/56	l, fl	–	0	0	..
		RUBIACEAE						
5578	*Cephalanthus occidentalis*	Jeff Davis Co., Tex.	8/55	l, tw, fr	–	0	0	..
5691	*Cephalanthus occidentalis*	Val Verde Co., Tex.	9/55	l, s, fl	–	0	++	..
5476	*Chiococca alba*	Monroe Co., Fla.	8/55	l, tw, fr	–	0	0	..
5468	*Chiococca pinetorum*	Dade Co., Fla.	8/55	l, tw	–	0	0	..
6406	*Genipa sp.*	Chiapas, Mexico	8/57	l, s, fr	–	0	0	..
5485	*Guettarda elliptica*	Monroe Co., Fla.	8/55	l, tw	–	0	0	..
5486	*Hamelia patens*	Monroe Co., Fla.	8/55	l, tw	–	0	+++	..
6005	*Mitchella repens*	Winsted, Conn.	6/56	l, s	–	0	0	..
5471	*Morinda royoc*	Monroe Co., Fla.	8/55	l, s, r, fr	–	0	0	..
5417	*Oldenlandia uniflora*	Leon Co., Fla.	8/55	l, s, r, fl	–	0	0	..
		RUTACEAE						

Accession No.	Species	Collection Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
6100	*Citrus limonia*	Chico, Calif.	10/56	l, tw	−	0	0	0
5159	*Citrus sinensis*	C. G. F.	9/54	fr	+	0	0	..
6158	*Citrus sinensis*	Chico, Calif.	11/56	l, tw	−	0	0	0
5294	*Esenbeckia hartmanii*	Sonora, Mexico	11/54	capsules	+	0	+	..
5295	*Esenbeckia hartmanii*	Sonora, Mexico	11/54	sd	−	0	0	..
6150	*Murraya paniculata*	C. G. F.	10/56	l, tw	−	0	0	0
5368	*Poncirus trifoliata*	Leon Co., Fla.	7/55	l, tw, fr	−	0	0	..
5373	*Ptelea trifoliata*	Gadsden Co., Fla.	7/55	l, s	−	0	0	..
6041	*Zanthoxylum Americanum*	Victor, N. Y.	7/56	l, s	−	0	0	..
6016	*Zanthoxylum clava-herculis*	Virginia	7/56	l, tw, fr	−	+	0	..
5525	*Zanthoxylum fagara*	Lee Co., Fla.	8/55	l, tw	−	0	++	..
5866	*Zanthoxylum sp.*	Atenas, Costa Rica	4/56	l, s	−	0	0	..
		SALICACEAE						
5240	*Salix babylonica*	Baltimore Co., Md.	10/54	l, s	−	0	0	..
5377	*Salix Caroliniana* (*Salix longipes*)	Gadsden Co., Fla.	7/55	l, tw	−	0	0	..
		SAPINDACEAE						
5170	*Cardiospermum integerrimum*	Glenn Dale, Md.	9/54	l, s	−	0	0	..
6408	*Cupania dentata*	Chiapas, Mexico	8/57	l, s, fl	−	0	0	..
6079A	*Dimocarpus longan* (*Nephelium longana*)	C. G. F.	9/56	l	−	0	0	+
6079B	*Dimocarpus longan* (*Nephelium longana*)	C. G. F.	9/56	tw	−	0	0	++
6079C	*Dimocarpus longan* (*Nephelium longana*)	C. G. F.	9/56	fr	−	0	0	0
5544	*Sapindus drummondii*	Brewster Co., Tex.	8/55	l, tw	−	0	0	..
5698	*Sapindus drummondii*	Terrell Co., Tex.	9/55	l, s, fr	−	0	0	..
5312	*Sapindus mukorossi*	Kabul, Afghanistan	2/55	sd, pod	+	0	0	.
5084	*Sapindus saponaria*	Pinellas Co., Fla.	8/54	l, s	−	0	0	..
6285	*Sapindus sp.*	Patulul, Guatemala	4/57	Hull	+	0	0	..
		SAPOTACEAE						
5477A	*Achras zapota* (*Sapota achras*)	Monroe Co., Fla.	8/55	l, tw	−	+	0	..
5477B	*Achras zapota* (*Sapota achras*)	Monroe Co., Fla.	8/55	fr	−	0	0	..
5392	*Bumelia lanuginosa*	Leon Co., Fla.	7/55	l, tw	−	+	0	..
5474	*Dipholis salicifolia*	Monroe Co., Fla.	8/55	l, s, fr	−	+	+	..
		SAXIFRAGACEAE						
5414	*Grossularia echinella*	Jefferson Co., Fla.	7/55	l, tw	−	0	0	..
5376	*Hydrangea quercifolia*	Gadsden Co., Fla.	7/55	l, tw	−	0	0	..
5930	*Mitella diphylla*	Frederick Co., Md.	5/56	l, s, r, fl	−	0	0	..
5959-O	*Peltiphyllum peltatum*	Calaveras Co., Calif.	6/57	w	−	0	0	..
6004	*Tiarella cordifolia*	Winsted, Conn.	6/56	l, s, r, fl	−	0	0	..
		SCHEUCHZERIACEAE						
5062	*Triglochin striata*	Levy Co., Fla.	8/54	l, s, fl	−	0	0	0
		SCROPHULARIACEAE						
5959	*Antirrhinum majus*	Baltimore Co., Md.	5/56	l, s, r, fl	−	0	0	..
5060	*Bacopa monnieri*	Levy Co., Fla.	8/54	w	−	0	0	0
6044	*Castilleja sp.*	Alberta, Canada	8/56	l, s	−	0	0	..

5039	*Chelone glabra*	Torrington, Conn.	8/54	l, s, fl	–	0	0	0	
5074	*Gerardia maritima*	Pinellas Co., Fla.	8/54	l, s, r, fl	–	0	0	0	
5037	*Gerardia paupercula*	Torrington, Conn.	8/54	l, s, fl, r	–	0	0	0	
5224	*Gerardia pinetorum*	Leon Co., Fla.	10/54	l, s, r, fl	–	0	0		..
5211	*Gerardia sp.*	Leon Co., Fla.	10/54	l, s, r, fl	–	+	0		..
5214	*Gerardia sp.*	Wakulla Co., Fla.	10/54	l, s, r, fl	–	0	0		..
5270	*Gerardia sp.*	Leon Co., Fla.	10/54	l, s, r	–	0	0		..
5707	*Leucophyllum frutescens*	Val Verde Co., Tex.	9/55	l, tw	–	0	0		..
5897-O	*Melampyrum lineare*	Perry Co., Pa.	8/56	l, s	–	0	0		..
5726	*Pentstemon ambiguus*	Ward Co., Tex.	9/55	w	–	0	0		..
6428A	*Pentstemon spectabilis*	Baja California, Mexico	9/57	sd	–	0	+		..
6428B	*Pentstemon spectabilis*	Baja California, Mexico	9/57	pod	–	0	++		..
5527	*Scoparia dulcis*	Pinellas Co., Fla.	8/55	l, s, r, fl	–	0	0		..
5124	*Scrophularia Marilandica*	York Co., Pa.	8/54	l, s, fr	–	0	0		..
5163	*Seymeria pectinata*	Leon Co., Fla.	9/54	l, s, r, fl	–	0	0		..
		SIMARUBACEAE							
6131	*Ailanthus altissima* (*A. cacodendron umbraculifera*)	Chico, Calif.	10/56	tw	–	0	0	0	
6164	*Ailanthus altissima* (*A. cacodendron*)	Chico, Calif.	11/56	l,	–	+	0	0	
5466	*Picramniapeltandra*	Dade Co., Fla.	8/55	l, tw	–	+	0		..
5898	*Quassia amara*	Guanacaste, Costa Rica	5/56	l, s, b	–	0	0		..
5469	*Suriana maritima*	Monroe Co., Fla.	8/55	l, tw	–	0	0		..
		SOLANACEAE							
6074	*Capsicum frutescens*	Baltimore Co., Md.	9/56	l, s, r, fr	–	0	0	0	
5585	*Chamaesaracha coronopus*	Brewster Co., Tex.	8/55	l, s, r, fl	–	0	0		..
6008	*Lycopersicum esculentum*	Baltimore Co., Md.	7/56	l, s, r	–	0	+		..
5168	*Methysticodendron amesianum*	Glenn Dale, Md.	9/54	l, s	–	0	+		..
5634	*Nicotiana trigonophylla*	Brewster Co., Tex.	8/55	l, s, fl	–	0	0		..
5383	*Physalis angulata*	Leon Co., Fla.	7/55	w	–	0	0		..
5505	*Physalis angustifolia*	Monroe Co., Fla.	8/55	w	–	0	0		..
5531	*Physalis elliottii*	Pinellas Co., Fla.	8/55	l, s, fr	–	0	0		..
5120	*Physalis ixocarpa*	York Co., Pa.	8/54	l, s, r, fr	–	0	0		..
5705	*Physalis lobata*	Terrell Co., Tex.	9/55	l, s, fr	–	0	0		..
5387	*Physalis pruinosa*	Leon Co., Fla.	7/55	w	–	0	+		..
5379	*Physalis Virginiana*	Liberty Co., Fla.	7/55	w	–	0	0		..
5760	*Physalis sp.*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0		..
5818	*Solanum acaule* var. *punae*	Sturgeon Bay, Wis.	2/56	w	–	+	0	0	
5819	*Solanum ajuscoense*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0	
5820	*Solanum andigenum cv. Collareja*	Sturgeon Bay, Wis.	2/56	w	–	+	0	0	
5821	*Solanum antipovichii*	Sturgeon Bay, Wis.	2/56	w	–	0	0		..
5507	*Solanum Bahamense*	Monroe Co., Fla.	8/55	w	–	0	0	0	
5822	*Solanum bulbocastanum* var. *glabrum*	Sturgeon Bay, Wis.	2/56	w	–	0	+	0	
5823	*Solanum chacoense*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0	
5827	*Solanum chacoense*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0	
5824	*Solanum commersonii*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0	
5825	*Solanum curtilobum cv. Shiri*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0	
5826	*Solanum demissum*	Brewster Co., Tex.	8/55	l, tw, r	–	0	0		..
5575	*Solanum elaeagnifolium*								

Accession No.	Species	Collection Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
5828	*Solanum fendleri*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5829	*Solanum gibberulosum*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5830	*Solanum gigantophyllum*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5386	*Solanum gracile*	Leon Co., Fla.	7/55	w	–	0	0	..
5831	*Solanum longipedicellatum*	Sturgeon Co., Wis.	2/56	w	–	0	0	0
5832	*Solanum macolae*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5833	*Solanum malinchense*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5834	*Solanum pinnatisectum* var. *heptazygum*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5835	*Solanum polyadenium*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5836	*Solanum polytrichon*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5582	*Solanum rostratum*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..
5837	*Solanum rybinii*	Sturgeon Bay, Wis.	2/56	w	–	0	0	+
5838	*Solanum saltense*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5839	*Solanum schickii*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5840	*Solanum stoloniferum*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5385	*Solanum torreyi*	Leon Co., Fla.	7/55	w	–	0	0	..
5841	*Solanum tlaxcalense*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5842	*Solanum tuberosum cv. Russet Sebago*	Sturgeon Bay, Wis.	2/56	w	–	0	0	0
5472	*Solanum verbascifolium*	Dade Co., Fla.	8/55	w	–	0	0	..
5843	*Solanum verrucosum* var. *spectabile*	Sturgeon Co., Wis.	2/56	w	–	0	0	0
5281	*Solanum sp.*	St. Johns Co., Fla.	11/54	l, s, r, fr	–	0	0	..
		STERCULIACEAE						
5369	*Firmiana platanifolia* (*Sterculia platanifolia*)	Leon Co., Fla.	7/55	l, tw	–	0	0	..
		TAMARICACEAE						
5752	*Tamarix sp.*	Pecos Co., Tex.	9/55	l, s	–	0	0	..
		TAXACEAE						
5025	*Taxus Canadensis*	Sandisfield, Mass.	8/54	l, s, r	–	0	0	+
		THYMELAEACEAE						
5374	*Dirca palustris*	Gadsden Co., Fla.	7/55	l, tw	–	0	0	..
		TILIACEAE						
5384	*Corchorus acutangulus*	Leon Co., Fla.	7/55	l, s, fr	–	0	0	..
		ULMACEAE						
5619	*Celtis reticulata*	Jeff Davis Co., Tex.	8/55	l, s, fl	–	0	0	..
5463	*Trema micrantha* (*Trema Floridana*)	Dade Co., Fla.	8/55	l, tw	–	0	0	..
		UMBELLIFERAE						
5996	*Aethusa cynapium*	Winsted, Conn.	6/56	l, s, r, fl	–	0	0	..
5999	*Angelica atropurpurea*	Winsted, Conn.	6/56	l, s, fl	–	0	0	..
6009	*Heracleum maximum* (*H. lanatum*)	Baltimore Co., Md.	7/56	l, s	–	0	0	..
5418	*Oxypolis greenmanii*	Calhoun Co., Fla.	8/55	w	–	0	0	..
5977	*Pastinaca sativa*	Carroll Co., Md.	6/56	l, s, fl	–	0	0	..
6049	*Petroselinum crispum* (*Apium crispum*)	Baltimore Co., Md.	8/56	l, s, r, fl	–	0	0	..
5249	*Sium sp.*	Liberty Co., Fla.	10/54	l, s, fl	–	0	0	..
5995	*Zizia aurea*	Winsted, Conn.	6/56	l, s, r, fl	–	0	0	..

		VERBENACEAE							
5593	*Aloysia ligustrina*	Jeff Davis Co., Tex.	8/55	l, fl, tw	–	0	0	..	
5071	*Avicennia nitida*	Pinellas Co., Fla.	8/54	l, s, fr	–	0	0	0	
5164	*Callicarpa Americana*	Leon Co., Fla.	9/54	l, s, fl	–	0	0	..	
5161	*Clerodendron indicum*	Leon Co., Fla.	9/54	l, s, r, fr	–	0	+	..	
6169	*Holmskioldia sanguinea*	C. G. F.	12/56	l, tw	–	0	0	0	
6427	*Lantana camara*	Superior, Ariz.	9/57	l, s	–	0	0	..	
5692	*Lantana horrida*	Val Verde Co., Tex.	9/55	l, s, fl	–	0	0	..	
5457	*Lantana involucrata*	Broward Co., Fla.	8/55	l, tw	–	+	0	..	
5408	*Lantana montevidensis*	Leon Co., Fla.	7/55	w	–	0	0	..	
5688	*Lippia berlandieri* (*L. graveolens*)	Val Verde Co., Tex.	9/55	l, s	–	0	0	..	
6325	*Lippia sp.*	Sonora, Mexico	5/57	b	–	0	0	..	
5503	*Stachytarpheta Jamaicensis*	Monroe Co., Fla.	8/55	w	–	0	0	..	
5606	*Verbena Neo Mexicana*	Jeff Davis Co., Tex.	8/55	l, s	–	0	0	..	
5570	*Verbena wrightii*	Jeff Davis Co., Tex.	8/55	l, s, r, fl	–	++	0	..	
5158	*Vitex agnus-castus*	Philadelphia, Pa.	9/54	l, sd	–	0	0	..	
5300A	*Vitex agnus-castus*	C. G. F.	12/54	l	–	++	0	0	
5300B	*Vitex agnus-castus*	C. G. F.	12/54	s	–	+	0	0	
5300C	*Vitex agnus-castus*	C. G. F.	12/54	fr	–	0	0	0	
5680-O	*Vitex agnus-castus*	Montgomery Co., Pa.	9/55	l, sd	–	0	0	..	
6180	*Vitex agnus-castus*	C. G. F.	1/57	l, s	–	0	0	0	
6199	*Vitex agnus-castus*	C. G. F.	1/57	l, s	–	0	0	0	
5301A	*Vitex altissima*	C. G. F.	11/54	l	–	++	0	0	
5301B	*Vitex altissima*	C. G. F.	11/54	s	–	++	0	0	
6197A	*Vitex altissima*	C. G. F.	1/57	l	–	++	0	++	
6197B	*Vitex altissima*	C. G. F.	1/57	tw	–	++	0	0	
6198A	*Vitex altissima*	C. G. F.	1/57	l	–	0	0	++	
6198B	*Vitex altissima*	C. G. F.	1/57	tw	–	++	0	0	
5302	*Vitex eylesii*	C. G. F.	11/54	l, s	–	0	0	0	
6195A	*Vitex parviflora*	C. G. F.	1/57	l	–	++	0	+	
6195B	*Vitex parviflora*	C. G. F.	1/57	tw	–	++	0	0	
6182	*Vitex parviflora*	C. G. F.	1/57	l, s, fr	–	+	0	0	
6151A	*Vitex parviflora*	C. G. F.	10/56	l	–	0	0	0	
6151B	*Vitex parviflora*	C. G. F.	10/56	tw	–	++	0	0	
5299A	*Vitex parviflora*	C. G. F.	11/54	l	–	++	0	0	
5299B	*Vitex parviflora*	C. G. F.	11/54	fr	–	++	0	0	
5299C	*Vitex parviflora*	C. G. F.	11/54	s	–	+	0	0	
5296A	*Vitex parviflora*	C. G. F.	11/54	l	–	++	0	0	
5296B	*Vitex parviflora*	C. G. F.	11/54	fr	–	0	0	0	
5296C	*Vitex parviflora*	C. G. F.	11/54	s	–	+	0	0	
5303	*Vitex quinata*	C. G. F.	11/54	l, s	–	0	0	0	
5305A	*Vitex sp.*	C. G. F.	11/54	l	–	++	0	0	
5305B	*Vitex sp.*	C. G. F.	11/54	s	–	++	0	0	
5305C	*Vitex sp.*	C. G. F.	11/54	fr	–	0	0	0	
5304A	*Vitex sp.*	C. G. F.	11/54	l	–	++	0	0	
5304B	*Vitex sp.*	C. G. F.	11/54	s	–	0	0	0	
5766	*Vitex sp.*	C. G. F.	11/55	l	–	0	0	..	
		VITACEAE							
5117	*Vitis labrusca*	Baltimore Co., Md.	8/54	l, s	–	0	0	..	

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis	Flavonoids	Alkaloids	Tannins
		ZINGIBERACEAE						
6414	*Costus bakeri*	Chiapas, Mexico	8/57	l, s	—	0	0	: :
		ZYGOPHYLLACEAE						
5137	*Balanites Aegyptiaca*	C. G. F.	9/54	l, s		0	0	: :
5609	*Kallstroemia hirsutissima*	Jeff Davis Co., Tex.	8/55	l, s		0	+	: :
5665	*Kallstroemia parviflora*	Brewster Co., Tex.	8/55	l, s, r, fl		0	0	: :
5617	*Larrea divaricata*	Jeff Davis Co., Tex.	8/55	l, s, fl		0	0	: :
5725	*Peganum harmala*	Ward Co., Tex.	8/55	l, s, fl		0	+	: :
5627	*Peganum Mexicanum*	Brewster Co., Tex	8/55	l, s, r, fl		0	+	: :
5671	*Tribulus terrestris*	Brewster Co., Tex.	8/55	l, s, fl		0	0	: :

of the collections in the present series gentrogenin and correllogenin account for about half of the total genin of 3 to 6%.

As was recently pointed out (10, 11) steroidal sapogenins in *Yucca* seem to be highly concentrated in the seed. Number 6371A is another example of it with 11.1%, all as sarsasapogenin. Another newly discovered genin, willagenin (12), reported from *Y. filifera*, appears again in this series in 6369, an unidentified species from Bacanora, Mexico.

The finding of ruscogenin and neoruscogenin in *Ruscus aculeatus* confirms the work of their discoverers (13).

Table II shows that an extremely small proportion of the plants in this series gave a test for saponin (hemolysis) and none of these contained steroidal sapogenins.

Plants containing flavonoids were also scarce.

There was, however, a high incidence of alkaloid bearing plants. Of the 1,000 species listed, 106, or 10%, contained alkaloids. Furthermore, of the 171 species in nine families collected in Texas 35, or 20%, contained alkaloids. Some 64 of these are new to the record.

Too few tests for tannins were made to warrant any generalizations.

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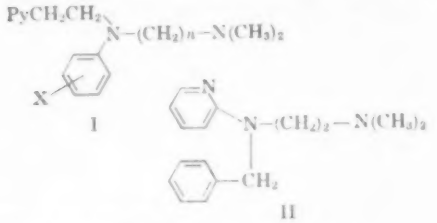
N-Dialkylaminoalkyl-N-(pyridylethyl)anilines

By SEYMOUR L. SHAPIRO, HAROLD SOLOWAY, EDWARD CHODOS, and LOUIS FREEDMAN

A series of N-dialkylaminoalkyl-N-(pyridylethyl)anilines has been prepared and on pharmacological evaluation significant hypotensive, anti-inflammatory, analgesic, and tranquillizing effects noted.

STRUCTURE-ACTIVITY relationships among the antihistamines and the phenothiazine type tranquillizer drugs (1) has indicated the criticality associated with small structural changes with such compounds. In this paper, a series of N-dialkylaminoalkyl-N-(pyridylethyl)anilines (I) envisioned as congeners of tripelennamine (II)



I

II

Py = 2-pyridyl, 2-pyridyl-5-ethyl, 4-pyridyl

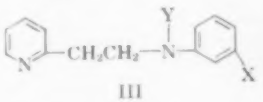
n = 2, 3

X = H, CH_3—, Cl, CH_3O—

has been synthesized and evaluated pharmacologically. In contrast to II, the pyridine ring of I is separated from the nitrogen by the ethylene group and the benzyl group of II has been herein replaced by a phenyl group directly attached to the nitrogen. In addition, substituents previously demonstrated to enhance activity in antihistamines and tranquillizing drugs have been introduced on the phenyl ring of I. In particular, the substitution of X = *m*-chloro, affords a group relationship paralleling that noted with the chlorine atom and ring nitrogen in chlorpromazine. The linking element between the nitrogens was varied to provide carbon chains, 2 and 3 atoms in length, whereas substitution on the more basic nitrogen was retained as the flat dimethylamino group (2).

A convenient synthetic route for I involved pyridylethylation of the N-dimethylaminoalkylanilines following the method of Levine (3). With these relatively high boiling pyridylethylated products, difficulties associated with reversibility (4) of the formed product to its reactants during distillation, seriously restricted the yields. See Table I.

A variety of other synthetic routes evaluated proved ineffective. Thus, pyridylethylanilines III, Y = H, could not be successfully alkylated with dialkylaminoalkyl halides following conventional procedures (5) or by fusion at elevated temperatures (6).



III

Y = H (IIIa), X = Cl
Y = —$COCH_2Cl$ (IIIb), X = Cl
—$COCH_2N(CH_3)_2$ (IIIc), X = Cl
—CH_2CH_2OH (IIId), X = H
—CH_2CH_2Cl (IIIe), X = H

The compound III, Y = H, gave the chloroacetamide (IIIb) which was converted to the dimethylacetamide (IIIc) which failed to yield the desired I on treatment with lithium aluminum hydride. Refractoriness to reduction of certain amides by lithium aluminum hydride has been noted by others (7–10).

As another alternative, the compound IIId did not yield the desired haloethyl derivative (IIIe) on treatment with thionyl chloride or 48% hydrobromic acid (11).

On pharmacological evaluation, the noted responses confirmed previous conclusions (1, 5) on the significance of structural variations on activity. Thus, hypotension was confined to the variants Py = 4-pyridyl with a 3+ response (12) being noted with compounds 12, 13, and 16 which had $LD_{min.}$ of 400, 500, and 350 mg./Kg. s.c., respectively.

Significant analgesia (13) was obtained with compounds 1, 7, and 13 with an ED_{50} of 90, 150, and 250 mg./Kg. s.c., respectively, all having X = *m*-chloro.

Interestingly, virtually all compounds evaluated for antihistamine effect showed no effect whatever with the exception of some potentiation with compound 7 and slight inhibition with compound 9.

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TABLE I.—N-DIALKYLAMINOALKYL-N-(PYRIDYLETHYL)ANILINES (I)

No.	X	n	M.P.,[a] °C. (R.S.)[b] or B.P., °C. (mm.)	Formula	Analyses, %[c] Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
				Py = 2-Pyridyl						
1	3-Cl	2	140–148 (0.03)	$C_{17}H_{22}ClN_3$	67.2	67.0	7.3	7.2	13.8	14.1
	[d]		180–183 (A)	$C_{29}H_{28}ClN_9O_{14}$	45.7	45.9	3.7	3.3	16.5	16.9
2	3-Cl	3	138 (0.02)	$C_{18}H_{24}ClN_3$	68.0	68.4	7.6	7.9	13.2	12.9
3	4-Cl	2	152–154 (0.09)	$C_{17}H_{22}ClN_3$	..	..	..	..	..	..
	[d]		142–145 (B)	$C_{29}H_{28}ClN_9O_{14}$	45.7	45.6	3.7	3.6	16.5	16.7
4	4-Cl	3	166–170 (0.05)	$C_{18}H_{24}ClN_3$	[e]	..	..	..	..	..
	[d]		160–163 (A)	$C_{30}H_{30}ClN_9O_{14}$	46.4	46.5	3.9	3.8	16.2	16.6
5	4-CH_3O	2	152–154 (0.07)	$C_{18}H_{23}N_3O$	72.2	72.1	8.4	8.4	14.0	14.3
	[d]		179–180 (C)	$C_{30}H_{31}N_9O_{15}$	..	..	..	..	16.6	16.9
				Py = 5-Ethyl-2-Pyridyl						
6	H	3	170–174 (0.05)	$C_{20}H_{29}N_3$	..	..	..	..	13.5	13.6
	[d]		160–163 (C)	$C_{32}H_{33}N_9O_{12}$	49.9	50.3	4.6	4.6	16.4	16.3
7	3-Cl	2	172–174 (0.20)	$C_{19}H_{26}ClN_3$	..	..	..	..	12.7	12.4
8	3-Cl	3	158–160 (0.03)	$C_{20}H_{28}ClN_3$	..	..	..	..	12.2	12.0
9	4-Cl	3	170–172 (0.02)	$C_{20}H_{28}ClN_3$	69.4	69.3	8.2	7.8	12.2	12.3
				Py = 4-Pyridyl						
10	H	2	154–156 (0.02)	$C_{17}H_{23}N_3$	..	..	..	..	15.6	15.5
	[d]		176–177 (D)	$C_{29}H_{29}N_9O_{14}$	47.9	48.1	4.0	4.2	17.3	17.6
11	2-Cl	3	156 (0.09)	$C_{18}H_{24}ClN_3$	..	..	..	..	13.2	12.7
12	3-Cl	2	164–168 (0.02)	$C_{17}H_{22}ClN_3$	67.2	67.0	7.3	7.3	13.8	14.0
13	3-Cl	3	178–180 (0.02)	$C_{18}H_{24}ClN_3$	..	..	..	..	13.2	12.8
	[d]		188–189 (D)	$C_{30}H_{29}ClN_9O_{14}$	46.4	46.4	3.9	4.2	16.2	15.6
14	4-Cl	2	168–170 (0.07)	$C_{17}H_{22}ClN_3$	..	..	..	..	13.8	13.8
	[d]		165–166 (D)	$C_{29}H_{28}ClN_9O_{14}$	45.7	45.7	3.7	3.8	16.5	16.7
15	4-Cl	3	168–170 (0.05)	$C_{18}H_{24}ClN_3$	..	..	..	..	13.2	13.0
16	3-CH_3	2	158–160 (0.05)	$C_{18}H_{25}N_3$	76.3	75.9	8.9	9.0	14.8	14.5
	[d]		178–179 (D)	$C_{30}H_{31}N_9O_{14}$	48.6	48.5	4.2	4.4	..	..

[a] Melting points were taken on the Fisher-Johns melting point apparatus and are uncorrected. [b] R.S. = recrystallizing solvent; A = water; B = acetone; C = methyl ethyl ketone; D = acetonitrile. [c] Analyses are by Weiler & Strauss, Oxford, England. [d] Dipicrate of compound immediately above. [e] Chlorine: Calcd.: 11.2; Found: 11.3.

Tranquillizing activity reflected by reduction in motor activity of rats (4) was noted with the following: the compound No./$LD_{min.}$/% reduction in activity/dosage tested: 6/250/59/50; 12/400/47/100; 14/250/69/100.

Tranquillizing activity was also manifest in the capacity of compound 10 ($LD_{min.}$ 200) and 16 to prevent audiogenic seizures in mice (14) with a PD_{50} of 72 and 75 mg./Kg.

Anti-inflammatory effectiveness (15) was noted with the following compounds showing 80–100% protection at 50 mg./Kg.: 1, 6, 9, 15, and 16, whereas the following showed 50–63% protection at 50 mg./Kg.: 4, 10, 12, 13, and 14. It is of interest that this latter category has representation in virtually all of the structural variants in the series.

EXPERIMENTAL

N - Dimethylaminoalkylanilines.—These intermediates were prepared by familiar procedures (16, 17) and are described in Table II.

N - Dimethylaminoethyl - N - (2 - [2 - pyridyl]-ethyl) - *p* - methoxyaniline (Table I, Compound 5).—A solution of 10.5 Gm. (0.1 mole) of 2-vinylpyridine, 19.4 Gm. (0.1 mole) of *N*-dimethylaminoethyl - *p* - methoxyaniline (Table II, compound 12), and 6.0 Gm. (0.1 mole) of acetic acid in 150 ml. of ethanol was heated under reflux for 24 hours. After removal of the solvent and basifying with aqueous sodium hydroxide, the separated oil was extracted with three 100-ml. portions of ether. The ether extracts were combined, dried (magnesium sulfate), filtered, the ether removed, and the residue distilled. After a forerun of 8.6 Gm. (44%) of the reactant aniline there was obtained 11.7 Gm.(40%) of product, boiling at 152–154° (0.07 mm.).

The other compounds of Table I were similarly prepared. The compound X = alkoxy gave the highest yield; those compounds with X = H or CH_3 afforded yields in the range 18–30%, whereas X = Cl gave yields of 2–12%. Many of the compounds were further characterized as their dipicrates.

N - (2 - [2 - Pyridyl]ethyl) - *m* - chloroaniline (IIIa) (3).—A mixture of 52.5 Gm. (0.5 mole) of 2-vinylpyridine, 63.8 Gm. (0.5 mole) of *m*-chloroaniline, 30.0 Gm. (0.5 mole) of glacial acetic acid in 125 ml. of methanol, after 8 hours reflux afforded 73.0 Gm. (64%) of crude product (m.p., 29–32°). On recrystallization (pentane) it melted at 54–55°.

Anal.—Calcd. for $C_{13}H_{13}ClN_2$: C, 67.1; H, 5.6; N, 12.0. Found: C, 67.2; H, 5.6; N, 12.1

N - (2 - [2 - Pyridyl]ethyl) - *p* - chloroaniline.—Prepared as above, melted at 61–62° (hexane).

Anal.—Calcd. for $C_{13}H_{13}ClN_2$: C, 67.1; H, 5.6; N, 12.0. Found: C, 66.8; H, 5.6; N, 11.9.

The picrate melted at 152–153° (ethanol).

Anal.—Calcd. for $C_{19}H_{16}ClN_5O_7$: C, 49.4; H, 3.5; N, 15.2. Found: C, 49.8; H, 3.7; N, 14.7.

N - (2 - [2 - Pyridyl]ethyl) - N - (*m* - chlorophenyl)chloroacetamide (IIIb).—A solution of 18.1 Gm. (0.16 mole) of chloroacetyl chloride in 250 ml. of acetonitrile was treated with 38 Gm. (0.16 mole) of N-(2-[2-pyridyl]ethyl)-*m*-chloroaniline, under cool-

TABLE II.—N-DIMETHYLAMINOALKYLANILINES

No.[a]	X	n	B.P., °C. (mm.)	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
				$X\text{-}C_6H_4NH(CH_2)_nN(CH_3)_2$						
1	H	2	86–88 (1)	$C_{10}H_{16}N_2$	73.1	73.1	9.8	10.6	17.1	16.7
2	H	3	119–122 (4)	$C_{11}H_{18}N_2$	...	...	...	...	15.7	15.7
3	2-Cl	2	158–160 (21)	$C_{10}H_{15}ClN_2$	60.5	60.7	7.6	7.9		
4	2-Cl	3	138 (4)	$C_{11}H_{17}ClN_2$	62.1	62.3	8.1	8.3	13.2	12.9
5	3-Cl	2	132–134 (4)	$C_{10}H_{15}ClN_2$	60.5	60.5	7.6	7.9	...	...
6	3-Cl	3	132–134 (1)	$C_{11}H_{17}ClN_2$	62.1	61.9	8.1	8.5	...	...
7[b]	4-Cl	2	157–162 (19)	....	...	...	...	...	...	...
8	4-Cl	3	147–149 (2)	$C_{11}H_{17}ClN_2$	...	...	...	...	13.3	13.2
9	3,4-diCl	2	102–104 (0.04)	$C_{10}H_{14}Cl_2N_2$	51.5	51.1	6.1	5.6	...	...
10	4-Br	3	110–114 (0.02)	$C_{11}H_{17}BrN_2$	...	...	...	...	10.9	10.8
11[b]	3-CH_3	2	111–113 (2)	...	...	...	...	...	...	...
12	4-CH$_3$O	2	133–137 (2)	$C_{11}H_{18}N_2O$	...	...	...	...	14.4	14.9

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ing, followed by a 2-hour reflux. The solvent was removed, the residue, in water, basified with 40% aqueous sodium hydroxide, and the product extracted with ether and dried (anhydrous magnesium sulfate). After removal of the solvent and recrystallization (hexane), there was obtained 25 Gm. (51%), m.p. 58–59°.

Anal.—Calcd. for $C_{15}H_{14}Cl_2N_2O$: C, 58.3; H, 4.6; N, 9.1. Found: C, 58.3; H, 4.6; N, 9.1.

The hydrochloride melted at 186–188° (ethanol-ether).

Anal.—Calcd. for $C_{15}H_{15}Cl_3N_2O$: C, 52.1; H, 4.4. Found: C, 52.0; H, 4.4.

The picrate melted at 145–147° (ethanol).

Anal.—Calcd. for $C_{21}H_{17}Cl_2N_5O_8$: C, 46.9; H, 3.2; N, 13.0. Found: C, 46.7; H, 3.5; N, 13.1.

N-(Dimethylaminoacetyl)-N-(2-[2-pyridyl]-ethyl)-*m*-chloroaniline (IIIc).—A mixture of N-(2-[2-pyridyl]ethyl)-N-(*m*-chlorophenyl)chloroacetamide (11.4 Gm., 0.035 mole) and 5 Gm. (0.10 mole) of dimethylamine in 50 ml. of ethanol was maintained in a pressure bottle at 60–70° for 6 hours. After cooling and removal of the ethanol, the residue, in 100 ml. of water, was basified with 40% aqueous sodium hydroxide, salted with potassium carbonate, and the separated oil extracted with ether. After drying (magnesium sulfate) and removal of the ether, the product distilled to give 5.7 Gm. (51%) b.p. 152–168° (0.05 mm.) (n_D^{30} 1.5624).

Anal.—Calcd. for $C_{17}H_{21}ClN_3O$: C, 64.0; H, 6.6; N, 13.2. Found: C, 64.3; H, 6.7; N, 12.7.

The dipicrate melted at 163–164° (ethanol).

Anal.—Calcd. for $C_{29}H_{27}ClN_9O_{15}$: C, 44.8; H, 3.5; N, 16.2. Found: C, 45.2; H, 3.6; N, 16.2.

Attempted reduction with lithium aluminum hydride in ether, after 14 hours reflux afforded only 52% of the reactant amide, IIIc: identified by analysis, and its dipicrate, not depressing the melting point of authentic dipicrate of IIIc.

N-(2-Hydroxyethyl)-N-(2-[2-pyridyl]-ethyl)aniline (IIId).—A mixture of 52.5 Gm. (0.5 mole) of 2-vinylpyridine, 68.5 Gm. (0.5 mole) of 2-anilinoethanol, 30.0 Gm. (0.5 mole) of glacial acetic acid in 125 ml. of ethanol, under 13-hour reflux was processed as detailed for the compounds of Table II. After work-up and removal of the ether, the crystalline residue was triturated with pentane and recrystallized (hexane) to give 26.5 Gm. (23%), melting at 67–69°.

Anal.—Calcd. for $C_{15}H_{18}N_2O$: C, 74.4; H, 7.5 N, 11.6. Found: C, 74.8; H, 6.9; N, 11.6.

An additional 20% of product was obtained from the pentane filtrate.

It is of interest that under these conditions no acetylation of the hydroxyl group was noted.

Attempts to convert the hydroxyl group to chlorine (thionyl chloride) or bromine (48% hydrobromic acid) afforded intractable tars.

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Leaf Alkaloids of *Rauwolfia nitida*

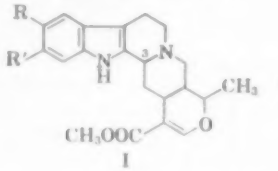
By RALPH SALKIN, NORMAN HOSANSKY, and ROBERT JARET

The leaves of *Rauwolfia nitida* Jacq. have yielded two new indole alkaloids, raunitidine, an 11-methoxy-δ-yohimbine, and rauniticine, an isomer of ajmalicine.

THE LITERATURE on alkaloids from *Rauwolfia* species has dealt almost exclusively with material of root origin while leaf alkaloids have received little attention.

From the leaves of *R. canescens*, Stoll, *et al.* (1), isolated, by chromatographic means, aricine, isoreserpinine, reserpiline, and isoreserpiline, in addition to α-yohimbine previously found by Chatterjee (2). Analysis by paper chromatography of the leaf alkaloids from *R. heterophylla* showed no reserpine and spots differing from those given by root material (3). Finally, from a study of the alkaloidal distribution in *R. serpentina*, Biswas (4) reported a 0.5% total alkaloid content for the leaves.

Korzun, *et al.* (5), have reported the presence of nine components in the weakly basic fraction from roots of *R. nitida* Jacq., a species indigenous to the West Indies and growing as shrubs or trees between 2 and 15 meters high (6). Recently we had occasion to investigate the leaves of this species and isolated from the weakly basic fraction two new alkaloids in addition to the known δ-yohimbines, reserpinine (0.06%), isoreserpinine (0.015%), and isoreserpiline (0.05%).



I

The major new alkaloid, raunitidine, m.p. 276–278°, $[\alpha]_D$ −30.6°, was obtained in 0.2% yield and shown by elemental and functional group analysis to be isomeric with reserpinine (I, R = H, R′ = OCH_3). The second alkaloid, rauniticine, m.p. 233–235°, $[\alpha]_D$ −6.6°, isolated in 0.04% yield, is isomeric with ajmalicine (I, R = R′ = H).

The ultraviolet spectra of the two bases were identical to those of reserpinine and ajmalicine, respectively. In their infrared spectra, bands characteristic of the —(CH_3OOC)C═C(OR)— chromophore appeared at 5.88 and 6.13 μ (7) while two distinct bands on the long wavelength side of the 3.4 μ band served to establish the 3α configuration (8) (Figs. 1 and 2).

Each alkaloid on alkaline hydrolysis gave methanol and the respective crystalline acid which could be re-esterified with methanolic hydrochloric acid, in each case, to the starting base.

Selenium dehydrogenation of rauniticine readily afforded alstyrine (9), identical with the sample prepared from ajmalicine, while raunitidine or its isomer reserpinine failed to afford a significant amount of characterizable material. Djerassi, *et al.* (7), encountered similar difficulties in attempting to characterize the decomposition products of tetraphylline, another of the 11-methoxy-δ-yohimbines.

Raunitidine under milder dehydrogenation conditions, e.g., mercuric acetate, yielded, as expected of a 3α epimer, 3-dehydroraunitidine. This latter was readily reduced with sodium borohydride to raunitidine.

Isomerization of yohimbine derivatives containing the unstable 3β configuration to the more stable 3α form with acetic anhydride or acetic acid has been reported (10). We have observed similar behavior in the δ-yohimbine series. Those compounds possessing the 3α configuration (i.e., ajmalicine, reserpinine, tetraphylline, and rauniticine) were recovered unchanged when refluxed with acetic anhydride, while those containing the 3β configuration (i.e., isoreserpinine and reserpiline) were epimerized to the more stable form.

Although 40% of starting material was recovered in the case of raunitidine, a new isomer with strong positive rotation, $[\alpha]_D$ +131°, was also isolated. This substance showed an unchanged ultraviolet spectrum and, in the infrared, the original bands at 5.88 and 6.13 μ of the enol ester chromophore as well as the characteristic bands for the 3α configuration (Fig. 3). Paper chromatography showed a single spot with an R_f different from all other known δ-yohimbines. The nature of this isomerization is being investigated.

Isoreserpinine isolated from the leaves of *R. canescens* by Stoll (1) as well as from *R. heterophylla* leaves in our laboratories had a specific rotation of −7° and showed a single spot on

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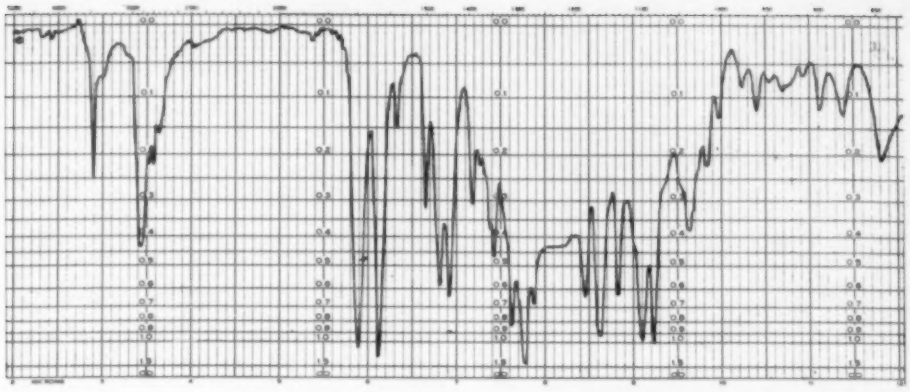

Fig. 1.—Chloroform solution spectra of raunitidine, 2%, 0.5-mm. cell.

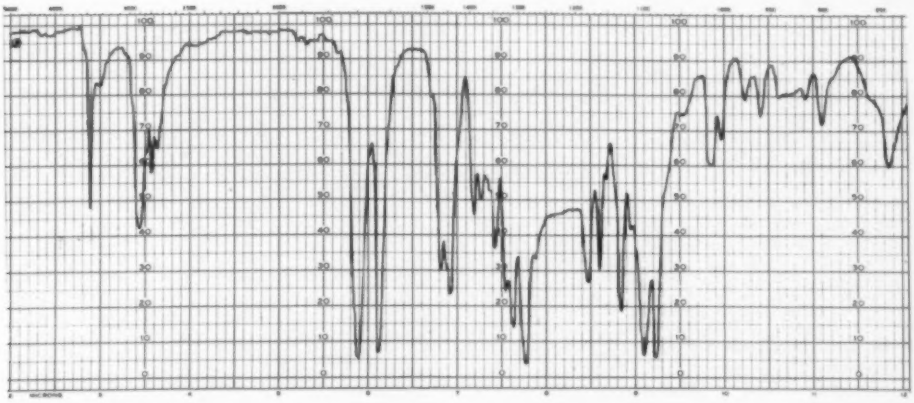

Fig. 2.—Chloroform solution spectra of rauniticine, 2% 0.5-mm. cell.

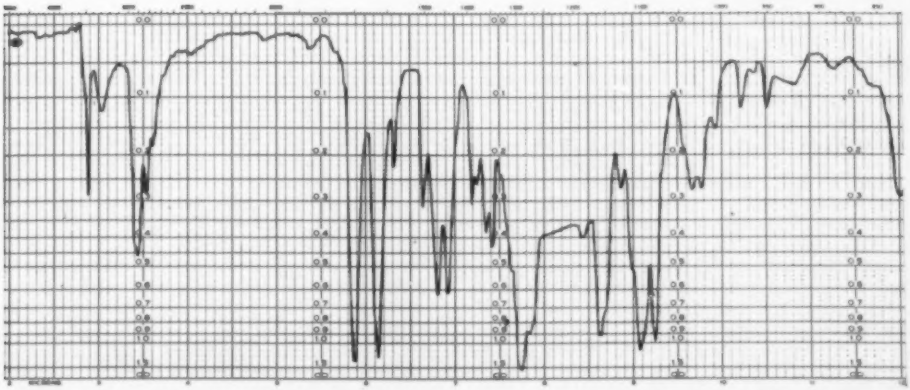

Fig. 3.—Chloroform solution spectra of isoraunitidine, 2%, 0.5-mm. cell.

paper. The comparable substance from *R. nitida* had a rotation of +7.5°. Infrared and chromatographic evidence suggested that this was caused by admixture with the positive rotating isomer of raunitidine. Since raunitidine is stable on long exposure to the acids used in the isolation procedure, the isomer is not an artifact but must exist as such in the plant.

In addition to the isolated bases, paper chromatography of the weakly basic fraction indicated the presence of aricine, reserpiline, and two other unknown alkaloids.

EXPERIMENTAL

Air-dried and powdered leaves (26 Kg.) of *R. nitida* were moistened with saturated sodium bicarbonate solution and extracted with benzene. The benzene extract was shaken with 0.5 *M* phosphoric acid. The bases were liberated from the acid by the addition of 10% ammonia and were extracted with 9:1 chloroform:methanol. Evaporation of the solvent left 702 Gm. (2.7%) of dark brown amorphous alkaloid fraction.

Raunitidine.—This fraction was refluxed 30 minutes with 4 L. methanol, the crystalline slurry chilled and filtered to give 78 Gm. crude raunitidine. Several crystallizations from alcohol of the hydrochloride from the crude base gave pure raunitidine hydrochloride as solvate, m.p. 230° (decompn.), $[\alpha]_D$ −108° (MeOH).

Anal.—Calcd. for $C_{23}H_{28}N_2O_4 \cdot HCl \cdot C_2H_5OH$: C, 61.99; H, 7.15; N, 6.03; Cl, 7.63. Found: C, 61.98; H, 7.38; N, 6.31; Cl, 7.85.

Conversion of the pure salt to base and crystallization from alcohol gave 52 Gm. raunitidine, m.p. 276–278°, $[\alpha]_D$ −30.6° (Py), −69.5° ($CHCl_3$); pKa = 5.4 (90% DMF); $\lambda_{max.}^{EtOH}$ 229 mμ (log ε 4.63), 298 mμ (log ε 3.80); $\lambda_{min.}^{EtOH}$ 282 mμ (log ε 3.63).

Anal.—Calcd. for $C_{22}H_{24}N_2O_4$: C, 69.09; H, 6.85; O, 16.75; N, 7.33; $2OCH_3$, 16.23; C-CH_3, 3.92. Found: C, 68.87; H, 6.71; O, 16.96; N, 7.31; OCH_3, 16.13; C-CH_3, 3.86.

The alkaloids recovered from the original methanol filtrate were dissolved in 12 L. 0.5 *M* phosphoric acid. The solution was adjusted to pH 4. The liberated alkaloids were extracted with chloroform and the chloroform solution, after washing with dilute carbonate and water, was evaporated to leave 205 Gm. of the weakly basic fraction. The alkaloids remaining in the pH 4 aqueous solution were recovered and set aside for further investigation.

Rauniticine.—From the weakly basic fraction, reserpinine (0.06%) and isoreserpiline (0.015%) were removed by means of their insoluble nitrates and impure isoreserpinine (0.015%), m.p. 221–223°; $[\alpha]_D$ +7.5° (Py), was recovered through its oxalate. The base from the oxalate mother liquor crystallized from methanol to give 25 Gm. crude rauniticine which still contained reserpinine, removed as its nitrate, and raunitidine, removed as its hydrochloride. The final mother liquors gave 10.4 Gm. (0.04%) pure rauniticine from methanol, m.p. 233–235°, $[\alpha]_D$ −6.6° (Py), −38.4° ($CHCl_3$); $\lambda_{max.}^{EtOH}$ 228 mμ (log ε 4.65), 282 mμ (log ε 3.91); $\lambda_{min.}^{EtOH}$ 267 mμ (log ε 3.86).

Anal.—Calcd. for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.87; O, 13.61; N, 7.95; OCH_3, 8.82; C-CH_3, 4.28. Found: C, 71.60; H, 6.98; O, 13.77; N, 8.14; OCH_3, 9.05; C-CH_3, 4.52.

Its hydrochloride crystallized from alcohol, m.p. 262–263°, $[\alpha]_D$ −67.5° (MeOH).

Anal.—Calcd. for $C_{21}H_{24}N_2O_3 \cdot HCl$: C, 64.85; H, 6.48; O, 12.34; N, 7.20; Cl, 9.11. Found: C, 64.73; H, 6.53; O, 12.47; N, 7.48; Cl, 9.20.

Alkaline Hydrolysis.—Raunitidine (5 Gm.) was refluxed 3 hours with 2 L. 2*N* potassium hydroxide in alcohol. The reaction mixture was worked up according to Stoll (1) to give 4.8 Gm. crude ammonium salt. The salt (2 Gm.) was digested with two 50-ml. portions of hot water, the clear solution adjusted to pH 7.1, and extracted 6 hours with ether. The residue, after evaporation of the ether, crystallized from acetone to give fine needles of raunitidic acid, m.p. 210°, $[\alpha]_D$ −69.5° (1:1 $CHCl_3$:MeOH); $\lambda_{max.}^{EtOH}$ 229 mμ (log ε 4.62), 298 mμ (log ε 3.79); $\lambda_{min.}^{EtOH}$ 281 mμ (log ε 3.61).

Anal.—Calcd. for $C_{21}H_{24}N_2O_4$: C, 68.46; H, 6.56; O, 17.37; N, 7.61. Found: C, 68.32; H, 6.68; O, 17.13; N, 7.80.

Treatment of the crude ammonium salt (1 Gm.) with 25 ml. 2% hydrochloric acid in methanol gave 250 mg. raunitidine, identified by mixed m.p., rotation, and infrared spectrum.

Similarly rauniticine gave rauniticic acid, crystallized from acetone, m.p. 227–229° (decompn.); $[\alpha]_D$ −37.0° (1:1 $CHCl_3$:MeOH).

Anal.—Calcd. for $C_{20}H_{22}N_2O_3$: C, 70.99; H, 6.55; O, 14.18; N, 8.28. Found: C, 70.89; H, 6.50; O, 14.42; N, 8.16.

Treatment with hydrochloric acid in MeOH as above gave rauniticine authenticated by mixed m.p., rotation, and infrared spectrum.

Selenium Dehydrogenation.—Rauniticine (1 Gm.) was intimately mixed with 1 Gm. selenium. The mixture was heated to 300°, maintained at that temperature for 20 minutes, cooled, mixed with sand, and extracted with ether for 8 hours. The ether residue was converted to 222 mg. picrate, the picrate decomposed to base, and the base chromatographed on alumina to give alstyrine from hexane, m.p. 110–111°; no melting point depression when admixed with authentic alstyrine prepared from ajmalicine.

Mercuric Acetate Dehydrogenation.—Raunitidine (1 Gm.) and 4.5 Gm. mercuric acetate in 50 ml. 10% acetic acid was heated at 80–90° for 2 hours. The crude perchlorate obtained after the usual work-up (8) was recrystallized from methanol to give bright yellow needles of the perchlorate of 3-dehydroraunitidine as dihydrate, m.p. 175° (decompn.); $[\alpha]_D$ −72.6° (MeOH); $\lambda_{max.}^{EtOH}$ 218 mμ (log ε 4.38), 390 mμ (log ε 4.41); inflection 260 mμ (log ε 3.88).

Anal.—Calcd. for $C_{22}H_{24}N_2O_4 \cdot HClO_4 \cdot 2H_2O$: C, 51.12; H, 5.66; N, 5.42; Cl, 6.86. Found: C, 51.34; H, 5.80; N, 5.72; Cl, 6.87.

The perchlorate in methanol was refluxed with sodium borohydride in the same solvent for 2 hours to give raunitidine, identified by mixed melting point and infrared spectrum.

Isomerization.—Raunitidine (1 Gm.) in 25 ml. acetic anhydride was refluxed for 18 hours. The reaction mixture was poured into ice. It was alkalinized with ammonia and the bases recovered with

chloroform. Methanol crystallization gave 380 mg. raunitidine. The mother liquors, after chromatography over alumina, gave another 75 mg. raunitidine and 260 mg. isoraunitidine from methanol, m.p. 259–261°; $[\alpha]_D$ +131° (Py); $\lambda_{max.}^{EtOH}$ 228 mμ (log ε 4.63); 298 mμ (log ε 3.80).

Anal.—Found: C, 68.90; H, 6.99; O, 16.90; N, 7.60.

Similarly 1 Gm. isoreserpinine gave 0.65 Gm. reserpinine, while 1 Gm. reserpiline gave 0.30 Gm. isoreserpiline.

Paper Chromatography.—The solvent systems were run on paper wetted with formamide. For the δ-yohimbines the mobile phase consisted of 5:1 heptane:methylethylketone, for the free acids 3:1 xylene:methylethylketone, and for the dehydro compound 2:1 xylene:methylethylketone containing 4% piperidine. The respective R_f values: reserpiline, 0.13; isoraunitidine, 0.17; isoreserpinine, 0.22; isoreserpiline, 0.24; ajmalicine, 0.53; raunitidine, 0.65; reserpinine, 0.78; aricine, 0.82; rauniticine, 0.92; raunitidinic acid, 0.53; rauniticic acid, 0.62; 3-dehydroraunitidine, 0.50.

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Investigation of the Applicability of Solid State Fluorescence to Pharmaceutical Analysis

By W. F. HEAD, Jr.†

Ten compounds were examined for their ability to fluoresce in the solid state as they occur in tablet form. Four of these compounds showed fluorescence which could be correlated with per cent composition of fluorophor. Particle size or compression variation did not affect fluorescence, although slight effects were noted due to the shape of the tablet surface and degree of smoothness. Instrumental parameters have been discussed.

THE GENERAL analytical scheme in the pharmaceutical industry depends initially on a sampling process to furnish a material representative of the entire substance. Usually this method works quite well but occasionally, due to nonhomogeneity of the material, a sample, within control limits, may be taken from a lot containing units which exceed these limits. The sample itself may contain units out of control but these may not be detected if averaged in an analysis with other correct units. Fortunately, with the aid of multiple specifications, multiple sampling, and process and intermediate control, this does not happen often.

Ideally, the analyst would like to assay each manufactured unit and be assured of 100% control. Continuous and complete control is being used today in some industries, notably petrochemical (1). The techniques used consist of continuous measurement of absorbance, conductivity, etc., of liquid or gas streams. There are also automatic analytical processes which still involve a sampling step. In this case, a sample, automatically and continuously collected, is analyzed rather than the entire material. Not many of these relatively simple and homogenous systems are found in the pharmaceutical industry.

A technique used to assay each individual unit of a production lot obviously must be nondestructive. Previous experiments have indicated that certain fluorophors can be detected in the solid state using a suitable fluorimeter. Solid state luminescence or fluorescence and solid state phosphorescence are not new; however, most quantitative fluorimetry has been performed in

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solution. If solid state fluorescence could be adapted to the direct measurement of the quantity of fluorophor within a range determined by control limits, it might then be possible to monitor continuously such items as tablets, capsules, powders, etc. Fluorescence would measure only per cent composition of fluorophor. For assurance of the proper dosage level, a consideration of the weight of each unit must also be made. In this work, weight has been taken as normal or "mean" and fluorophor content expressed as per cent composition. In actual practice, such a technique would require measurement of the weight of each unit as well as measurement of fluorescence. An additional assumption made is that each unit is homogeneous within itself; that is, the surface and interior compositions are the same. Other similar techniques, utilizing X-ray fluorescence or diffraction or "dense media" absorption spectrophotometry (2) could possibly be applied to nonfluorescent materials.

EXPERIMENTAL

Instrumentation and Equipment.—The optical system of an Aminco-Bowman spectrophotofluorometer was used in all phases of this work. When working at high signal levels, a Brown recorder with a continuously variable span of 1 to 21 mv. was employed with the photometer output of the spectrophotofluorometer. At lower signal levels, the "XY" recorder furnished with the optical unit was used. For tablet preparation, three-eighths inch standard concave punches and die and one-half inch flat punches and die were used with a Carver laboratory press. Optical filters used were made by the G. K. Turner Co., Palo Alto, Calif.

Methods.—In determining activation and emission wavelengths, the emission wavelength was scanned (200 to 800 mμ) while the activation monochromator was manually advanced 20 mμ after each complete scan. When a fluorescent peak was observed, the emission monochromator was held steady and the activation wavelength varied slowly until maximum signal was obtained.

Tablet samples were placed in the cell compartment diagonally so that the incident beam was at an angle of 45° with the face of the tablet. The emission monochromator slit was also at a 45° angle with respect to the tablet surface and 90° to the incident beam. A black cardboard tablet holder was initially used, but the tablets could not be repositioned accurately on either the vertical or horizontal axis. As a result, fluorescence readings could not be reproduced. An aluminum holder was designed, in which the tablets were held firmly against a front plate by a clip. A square cut into the front of the holder allowed a portion of the surface of the tablet to be exposed to the incident beam. The holder was painted with flat black paint to minimize reflection and it fit snugly in the cell chamber so that vertical and horizontal alignment could be reproduced accurately.

Much of this work was done using the Mosely "XY" recorder furnished with the spectrophotofluorometer. However, with large emission signals, X and Y axis interaction caused fluorescent peaks to be distorted. It was desirable to amplify these large signals even more and "buck out" most of the signal with a constant voltage source in order to detect any small difference between two large electrical potentials. To accomplish this and to avoid distortion, the Brown multispan recorder was used. The amplified signal was fed to the potentiometer circuit of the recorder and the time base axis was representative of emission scanning. Distortion of peaks was not possible since there is only one signal axis. An external potentiometer bucking signal was fed into the recorder with the fluorescence signal so that only the very tops of the extremely large peaks were traced on the chart. The block diagram of this system is shown in Fig. 1.

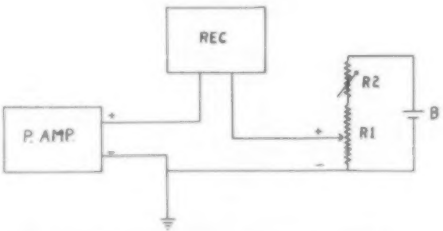

Fig. 1.—Block diagram of potentiometric signal biasing system. *P. AMP.*, Photometer amplifier; *REC*, Brown recorder; *R1*, 100 ohms; *R2*, 5K ohms; *B*, 5.35 v. mercury battery.

The sample may be thought of as a first surface mirror in the optical path, reflecting the activation radiation directly into the emission monochromator. When the emission is scanend, an extremely large signal is obtained when the monochromators match and again when the emission monochromator is at a value twice that of the activation wavelength being used (second-order diffraction of the activation wavelength). The fluorescent peak usually occurs between these two large signals and often can only be seen as a shoulder on one of the large peaks. To remove these activation cross-over signals, interference filters, closely matching the fluorescent emission, or cut-off filters, opaque to the activation wavelength and transparent to the emission wavelength and longer wavelengths, were placed in front of the photomultiplier tube.

Examination of Fluorophors.—Sodium salicylate, quinine sulfate, and riboflavin were chosen for initial observation on the basis of their excellent solution fluorophor characteristics. Each of these materials was made into tablets containing 0, 25, 50, 75, and 100% of active material, using a mixture of ethyl cellulose, potassium chloride, and about 1% sodium stearate as a filler. This filler represents some of the ingredients used as binders, lubricants, etc., in tablets. The tablets were made in the flat pellet die at a pressure of 20,000 pounds.

Sodium salicylate at an activation wavelength of 310 mμ and an emission of 415 mμ (*A* 310, *E* 415), gave a very large signal which, even at lowest photometer sensitivity of amplification, was unmeasurable. By placing black paper diaphragms

in front of the tablet, the area of tablet exposed to the light beam was decreased from 16 sq. mm. to approximately 0.7 sq. mm. This decreased the signal so that the various fluorescent intensities could be measured. A plot of fluorescence *vs.* per cent composition is not linear, but the slope decreases as concentration increases. This behavior is similar to self-quenching in liquid fluorophors at higher concentration. The spectrum and the emission intensity *vs.* concentration curve are shown in Figs. 2 and 3.

Riboflavin tablets had a fluorescence (*A* 385, *E* 610) which was almost linear with per cent compositions; however, the emission was only a small fraction of that observed with sodium salicylate. When activation wavelengths of the usual solution values (*A* 420, 445, 475) were used, the emission from 580 mμ to 600 mμ was insignificant.

Quinine sulfate did not exhibit significant fluorescence at any activation or emission wavelength.

Since only one of the three compounds, all being extremely strong solution fluorophors, showed good solid state fluorescence, several different commercial tablets were selected and scanned (without filters) for fluorescence. These included acetylsalicylic acid,[1] diethylstilbestrol (DES), aminophylline, ascorbic acid, nicotinic acid, nicotinamide, and isonicotinyl hydrazide (INH). A blank tablet emission spectrum was obtained by using a tablet containing 1% stearate in a mixture of equal parts potassium chloride, ethyl cellulose, and starch. Of these products only acetylsalicylic acid (*A* 275, *E* 330) and DES (*A* 250, *E* 350) showed good fluorescent peaks.

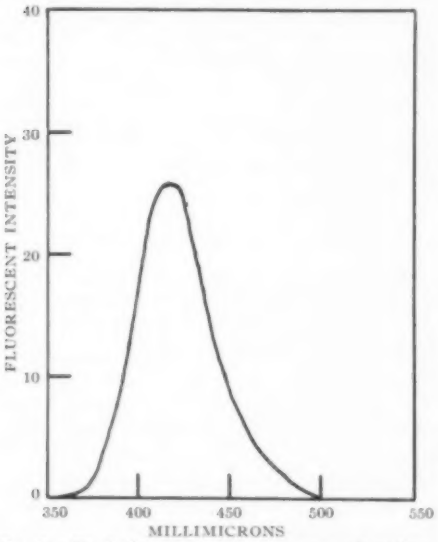

Fig. 2.—Typical fluorescence spectrum of sodium salicylate. Activation 310 mμ.

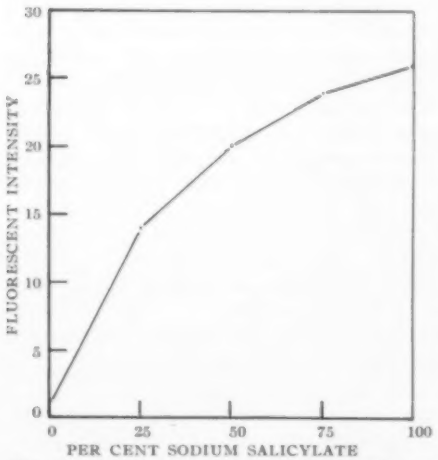

Fig. 3.—Fluorescence *vs.* per cent composition of sodium salicylate. Activation 310 mμ, emission 415 mμ.

A tabulation of almost 3,000 fluorescent compounds is given in DeMent's monograph on fluorescence (3). Most of the compounds used in this study are listed as being fluorescent (blue to violet) in the original state when activation light from a mercury arc lamp (full spectrum) is used. Therefore, at an activation of 365 mμ, and using a Turner No. 110-816, 415 mμ cut-off filter (415 mμ C.O.), all tablets were re-examined at a higher sensitivity. These tablets were made from pure materials to avoid any extraneous peaks due to excipients, binders, etc. Again, no fluorescence was observed except with acetylsalicylic acid and DES.

Prior to fluorescence, there must be absorption of radiation. Therefore, the emission of all compounds was scanned using activation wavelengths corresponding to their absorption maxima. The only fluorophors found were the same ones previously detected. Only the 370 mμ absorption band of riboflavin produced fluorescence. The other absorption peaks (265, 445, 475 mμ) did not yield fluorescent peaks at all or the emission observed was poor and did not correspond to per cent composition of riboflavin.

Sodium salicylate, acetylsalicylic acid, and DES were good fluorophors; riboflavin was a poor to moderate fluorophor. It is interesting that DES irradiation (250 mμ) produces a yellow compound and the fluorescent intensity diminishes with time. It is necessary to read the emission quickly and avoid overexposure. The optimum conditions for these fluorophors are listed in Table I.

Effect of Tablet Variables on Fluorescence.—Several 10-gr. commercial sodium salicylate tablets were ground and sieved into powders of 40–80 mesh and greater than 80 mesh. Tablets were then made from these powders, varying the compression, particle size, and shape of the tablet in turn. A standard concave tablet was compared to a flat tablet, holding pressure and particle size constant at 10,000 lb. and 40–80 mesh, respectively. Flat tablets made at 10,000 and 20,000 lb. (using 40–80 mesh powder) and using both particle sizes (at 10,000 lb.) were used for testing the effect of compression and particle size. All tablets had the same per cent composition of fluorophor.

[1] Marketed as A.S.A. by Eli Lilly and Co., Indianapolis 6, Ind.

Table I.—Spectral Characteristics of Fluorophors

Fluorophor	Activation, mμ	Emission, mμ	Filter
Sodium salicylate	310	415	Turner No. 110-816 415 mμ C.O.
Riboflavin	370	595	Turner No. 110-820 595 mμ C.O.
DES	250	350	Turner No. 110-811 360 mμ interference filter
Acetylsalicylic acid	275	330	1/4 mil Mylar film A's 330 mμ—0.012 A's 275 mμ—∞

Rather than use diaphragms to decrease the sodium salicylate fluorescence, a less effective activation wavelength (250 mμ) was used. This allows the entire sample (16 sq. mm.) to be viewed and differences in fluorescence are more likely to be detected. This is especially true in the case of tablet shape variation. Under these conditions, fluorescent intensities were the same for all tablets.

These tablets were examined again at a higher sensitivity using 310 mμ activation, a higher photometer amplification, and the signal biasing system with the Brown recorder. Again, no difference in fluorescent intensities was observed. However, under these conditions, there was too much signal and amplification for the photometer to function within limits. The output circuit of the amplifier has a current controlling network to prevent overloads and, apparently, the difference in tablet fluorescence, if present, could not be detected because the signals could be amplified only to a certain maximum.

Using the 250 mμ activation again to decrease the fluorescence, but increasing photometer amplification and biasing the signal before it reached the Brown recorder (the photometer operating within its limits), a difference in fluorescence was observed between standard concave tablets and flat tablets. Differences in fluorescence due to particle size or compression variation were not detected. The rounded tablets gave a slightly less intense signal than did flat tablets.

Roughness or smoothness of tablet surface also affect signal intensity. A flat tablet, roughed by filing, gives a stronger emission signal. This effect was not a large one even when comparing a completely dull surface to an almost mirror-like surface. The fluorescence differences due to surface shape and smoothness do not pose a serious problem since tablets of a given lot do not vary greatly in surface character. In addition, the magnitude of signal difference is only approximately 1 to 4 mv. in a 1-volt signal.

Small Variations of Fluorophor Concentration.—Sodium salicylate, riboflavin, acetylsalicylic acid, and DES fluorescence have been found to be governed by per cent composition over a wide range of concentration. For purposes of determining whether tablets from a production line are within limits, the technique must be able to distinguish between a very small range of fluorophor concentration.

Commercial tablets of the four fluorophors were ground to a powder and accurately weighed quantities of active material were added to give a slightly higher per cent fluorophor composition. The amounts of material added were determined by the permissible variation allowed in the specifications for each tablet. For example, a table having a range of 50 to 55% composition (control limits) would be ground and its mean of 52.5% assumed. To two portions of this material, enough fluorophor would be added to bring the composition up to 55 and 57.5%. Recompression of original tablet material and tablet material plus added fluorophor was carried out at 10,000 lb. using both a standard concave and a flat punch and die.

Differences in fluorescence were observed when the signal biasing system and maximum permissible amplification were used. However, these differences were random and not in accord with per cent composition. This variation was due to the normal amplifier, phototube, and lamp instability and was also present with liquid fluorophor systems under these conditions. It was necessary to make several readings to establish an average. In doing this, no significant fluorescence difference was observed for slight fluorophor variation in any of the four substances.

SUMMARY AND CONCLUSIONS

Solution fluorophors may or may not exhibit fluorescence in the solid state. The reasons for this behavior are possibly concerned with the existence of certain resonance forms or the ability to form resonance species in the solid condition. Of 10 known solution fluorophors examined, four showed fluorescence in the original state. This fluorescence was related to concentration of fluorophor and was not affected by particle size or compression. Slight effects were produced by variation of tablet shape or degree of surface smoothness. Fluorescence variations due to slight differences in fluorophor content could not be detected. The ease of such detection will depend on the slope of the fluorescence *vs.* composition curve in the particular region under consideration, becoming progressively harder as the slope approaches zero. The strong signals could be amplified many times, then biased, but any difference in signals could be lost in instrumental instability. If the photometer amplifier used in this work were replaced by an extremely high output, high stability amplifier, a difference in emission might possibly be detected. Before realization of complete product control, much more research must be done, especially in instrumentation for this purpose, and in other techniques in addition to fluorescence.

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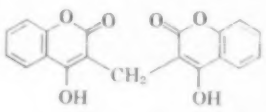
Crystallography of Bishydroxycoumarin

By HARRY A. ROSE and JOHN G. WILLIAMS

BISHYDROXYCOUMARIN or 3,3′-methylenebis-[4-hydroxy-1,2-benzopyrone] is used in medicine as an anticoagulant. Data are presented here which permit the identification of this compound by crystallographic methods.

The compound is represented by the following structural formula



Crystals suitable for crystallographic work may be obtained by crystallization from a hot, saturated benzene solution in spite of the low solubility in that solvent. The crystals are short monoclinic rods elongated parallel to the a axis, showing the 011 faces and closed by the 100 faces. Occasionally a small 010 face may be seen.

The X-ray powder diffraction data were obtained using copper radiation and nickel filter. A wavelength value of 1.5405 Å. was used in the calculations. The indexing of the powder pattern was done on the basis of single crystal rotation patterns around both the a and b axes. Tables I, II, and III give the X-ray diffraction data, optical crystallographic data, and X-ray powder diffraction data, respectively.

TABLE I. X-RAY DIFFRACTION DATA

Unit cell dimensions	a_o = 11.76 Å.; b_o = 15.16 Å.; c_o = 8.47 Å.
Formula weights per cell	4
Formula weight	336.29
Density	1.495 Gm./ml. (displacement), 1.497 Gm./ml. (X-ray)
β Angle	98°10′
Axial ratio	$a : b : c$ = 0.775:1:0.5587
Space group	C_{2h}^2 – $P2_1/m$

TABLE II.—OPTICAL CRYSTALLOGRAPHIC DATA

Refractive indexes	α = 1.508, $\beta > 1.70$, $\gamma > 1.70$
Optic axial angle	(–), $2V$ large
Orientation	$\alpha \wedge a$ = 53° in acute β, $\gamma = b$

TABLE III.—X-RAY POWDER DIFFRACTION DATA

d	I/I_1	hkl	d (calcd.)
8.38	0.40	001	8.38
7.46	0.60b	020, 011	7.58, 7.33
6.41	0.04	120	6.35
5.82	0.40	111, 200	5.84, 5.82
5.55	0.08	021	5.62
5.35	0.08	210	5.43
5.15	0.12	201	5.13
4.62	0.16	220	4.72
4.27	0.30b	211, 22$\bar{1}$, 002	4.31, 4.25, 4.19
4.05	0.16	012	4.04
3.79	0.12	040	3.79
3.68	0.30	20$\bar{2}$, 022	3.65, 3.65
3.57	1.00	21$\bar{2}$	3,58
3.31	0.60	22$\bar{2}$	3.29
3.20	0.16	032, 202	3.23, 3.19
2.97	0.40	23$\bar{2}$	2.97
2.90	0.04	40$\bar{1}$	2.91
2.79	0.04	003	2,79
2.70	0.04	23$\bar{2}$	2.70
2.63	0.04	02$\bar{3}$	2.62
2.59	0.04	40$\bar{2}$	2.57
2.52	0.04	25$\bar{1}$	2.51
2.47	0.04		
2.38	0,04		
2.34	0.04		
2.30	0.04		
2.24	0.02		
2.15	0.04		
2.06	0.04		

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Effects of Gibberellic Acid on *Nicotiana rustica* var. *Brasilia* Plants

By D. P. CAREW

A study was made of the effect of gibberellic acid on the growth of tobacco plants and the accumulation of nicotine. While gibberellic acid increased the rate of growth and the total chlorophyll content, a decrease in nicotine content was observed.

THE EFFECTS of gibberellic acid (G.A.) on plants are well known. There have been numerous reports concerning the effects of G.A. on various plant physiological processes such as cell division, stem elongation, and flowering (1, 2). However, reports concerning the effect of G.A. on the accumulation of secondary plant constituents have been much less numerous (3–5). In this investigation, studies were conducted to determine the effects of G.A. on *Nicotiana rustica* var. *Brasilia* L. plants.

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EXPERIMENTAL

The *Nicotiana* plants were grown from seed (*Nicotiana rustica* var. *Brasilia*) in a greenhouse. The seed was sown on May 1 in flats and later transferred to individual pots. When the plants were nine weeks old they were divided into three groups. Two groups, consisting of 14 plants, underwent treatment with G.A. and the third group of seven plants served as a control.

Freshly prepared aqueous solutions of G.A. containing 0.1% Tween 80 were employed. The levels of treatment were 25 and 100 p.p.m. Contrary to the common procedure of simply dipping or spraying the plant or plant part with a solution of G.A., in this experiment a known volume of a G.A. solution of definite strength was applied. In each case treatment was accomplished by spraying the leaves of the plant with 25 ml. of the appropriate solution contained in an atomizer. The control plants were sprayed with 25 ml. of distilled water containing 0.1% Tween 80. The treated plants each received three treatments spaced at 10-day intervals.

The general appearance and height were recorded at periodic intervals throughout the experiment. The length and width of the lamina of the five lower leaves of each plant were measured periodically. The plants were harvested two weeks after the final treatment and at that time the leaves were separated from the main axis and the fresh weight of the leaves and the stems was determined. The leaves and stems were then separately broken up and dried in a forced-air circulating oven at 40° for 96 hours and the dry weights were determined. The dried leaf and stem material was then stored in vacuum desiccators for subsequent chlorophyll and nicotine analysis.

The procedure used for the determination of total chlorophyll was that of the A.O.A.C. (6). The chlorophyll content of the leaves of control plants was used as a standard. Using various dilutions, as outlined in the A.O.A.C. procedure, the total chlorophyll content of the treated and untreated plants was determined.

Dried leaf and stem tissue was analyzed separately for nicotine as follows: the dried leaves or stems of individual plants were ground in a Wiley mill to a No. 20 powder. The ground tissue was then thoroughly moistened with 30% sodium hydroxide solution, air dried, and then extracted with anhydrous ether for 24 hours in a Soxhlet extractor. After 24 hours, the ether extract was removed, fresh solvent added, and the tissue was again extracted for 24 hours. The ether extracts were combined and concentrated to a volume of 150 ml. This concentrate was transferred to a separator where it was extracted six to eight times with 75-ml. portions of 15% sulfuric acid. The aqueous extracts were then combined, cooled, and made distinctly alkaline with 30% sodium hydroxide solution. This alkaline mixture was extracted six times with separate 125-ml. volumes of ether. The extracts were combined and the ether was removed. The residue was dissolved in 95% ethanol and allowed to stand for 24 hours to allow for salt precipitation. The alcoholic solution of nicotine was concentrated to a volume of 10 ml. on a water bath. To this concentrate was added a saturated solution of picric acid in 95% ethanol and the dipicrate salt of nicotine was formed. This compound was recrystallized from ethanol dried, and weighed. In each case the compound melted at a temperature of 223–225°. The nicotine content of the control plants was used as a basis for determining any increase or decrease in nicotine content of the treated plants.

RESULTS AND DISCUSSION

The treated plants responded to G.A. in a typical manner. They increased in height rapidly following the first and second applications. Most of the increase was internodal. While the treated plants exhibited an initial response to G.A., as the growing season continued their rate of growth was greatly reduced. The increase in height appeared to be due to increased cell elongation in the stem tissue, a phenomenon very common to plants treated with G.A. There was no significant difference between the number of leaves formed on the control and treated plants. The height of all plants was determined periodically and the growth rates are recorded in Table I.

Table I.—Height of Treated and Control Plants

	Initial Height[a]	10 Weeks	11 Weeks	12 Weeks	Final Height
25 p.p.m.	33.0	53.0	74.25	77.0	81.25
100 p.p.m.	34.25	54.5	83.25	87.25	88.75
Controls	31.5	36.0	45.0	52.5	58.0

[a] Measurements are in cm. and each figure represents the average of the plants in the group. The initial height was measured on the day of the first treatment and the final height was recorded on the day of harvest.

A comparison of the fresh and dry weights of the leaves and stems further illustrates the tendency of G.A. to promote stem elongation. As indicated in Table II the fresh and dry weight of stem tissue of control plants is much less than for the treated plants.

Table II.—Fresh and Dry Weight[a] of Treated and Control Plant Parts

	Leaves		Stems	
	Fresh	Dry	Fresh	Dry
25 p.p.m.	154.2	19.4	80.0	27.9
100 p.p.m.	166.6	21.2	98.1	33.3
Controls	160.0	20.5	52.5	15.0

[a] Weights are in Gm. and each figure represents the average of the plants in a group.

At the time of harvest the results of leaf surface measurements revealed that there was no significant difference between the size of the leaf surfaces of the two levels of treated plants. However, the treated plants possessed leaves which were on the average 6 cm.^{2} greater in surface area than those of the control plants.

While plants under the influence of G.A. commonly exhibit a chlorotic effect it has been previously noted that these plants may actually contain an increased amount of chlorophyll (7). The treated plants in this experiment exhibited a mild chlorosis as well as occasional evidence of sun-scalding. The results of the total chlorophyll determination in the treated and control groups revealed no significant

difference in the amount of chlorophyll present in the two groups of treated plants. However, the treated plants were found to possess 10% more total chlorophyll than the control group.

In determining whether there was any increase or decrease in nicotine content in the leaves and stems of the treated and control groups, the nicotine found in the control plants was considered as 100%. By comparison, it was found that the leaves of plants treated with 25 p.p.m. of G.A. contained 79.2% nicotine, and the leaves of plants treated with 100 p.p.m. G.A. contained 93.2% nicotine. Previous workers have also reported a decrease in alkaloid concentration in G.A.-treated plants (8–10). The nicotine content of the stems of both treated and control groups was very low and there was, in fact, no significant difference between the amount present in the two groups.

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Communications

Magnetic Iron Oxide for Use in Tablet Coating

Sir:

Rednick, *et al.* (1), reported the use of reduced iron as an aid in pan coating small batches of tablets. The procedure involved incorporating a small amount of the iron in the placebo tablets, combining both the active and placebo tablets (having the same dimensions), coating the mixed batch of tablets and finally separating the active from placebo tablets by passing them through a magnetic selector.

Since that time a high permeability magnetic iron oxide (an oxygen saturated iron oxide—Fe_2O_3) has been made available to us.[1] The lower chemical reactivity of iron oxide makes its use preferable to that of reduced iron. This magnetic iron oxide reportedly has a higher permeability than reduced iron; however, no attempt was made to verify this.

The magnetic iron oxide was incorporated into two lots of tablets (containing 5 mg. each) using a wet granulation method for one and a slugging procedure for the other. To 100 tablets of each formula was added 20,000 placebos of identical size and shape but without the iron oxide. Both batches where then sugar coated, making the iron oxide tablets indistinguishable from the placebo tablets.

The material was then passed through a magnetic selecting machine which removed the iron oxide tablets, thus demonstrating that magnetic iron oxide is an adequate substitute for reduced iron.

(1) Rednick, A. B., Nicholson, A. E., and Tucker, S. J., THIS JOURNAL, **50**, 174(1961).

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[1] Wright Industries Inc., 412 55th St., Brooklyn 20, N. Y. Available in research quantities only.

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ERRATUM

In the paper titled "Rate of Release of Medicaments from Ointment Bases Containing Drugs in Suspension" (1), the last equation should read

$$\frac{dQ}{dt} = \sqrt{\frac{ADC_s}{2t}}$$

rather than

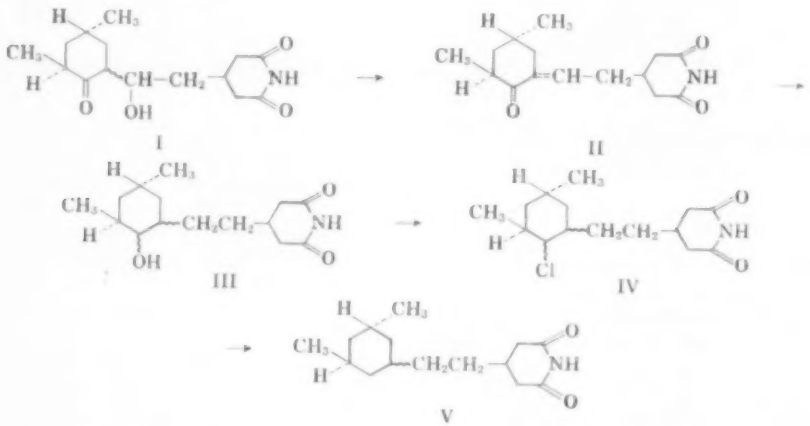
$$\frac{dQ}{dt} = \frac{ADC_s}{2t}$$

(1) Higuchi, T., THIS JOURNAL, **50**, 874(1961).

Determination of the Absolute Configuration of Cycloheximide

Sir:

Cycloheximide, an antibiotic obtained from *Streptomyces griseus*, is of particular interest because of its antifungal and anticancer activity. Recently, the absolute configuration of the methyl group gamma to the keto group in cycloheximide (I) was determined by relating this center to D-glyceraldehyde using reactions which could not have caused an isomerization (1).



I II III IV V

We have now determined the absolute configuration of the methyl group alpha to the keto group of cycloheximide by the following sequence: treatment of cycloheximide (I) with phosphorus pentoxide in benzene gave anhydrocycloheximide (II), which on hydrogenation with a platinum catalyst in acetic acid gave tetrahydroanhydrocycloheximide (III) (2). When tetrahydroanhydrocycloheximide (III) was allowed to react with thionyl chloride in dioxane solution, a good yield of the chloro compound (IV) was obtained (m.p. 136–138°).

Anal.—Calcd. for $C_{15}H_{24}ClNO_2$: C, 63.03; H, 8.46; N, 4.90; Cl, 12.40. Found: C, 63.29; H, 8.29; N, 5.05; Cl, 12.14.

Treatment of compound IV with zinc dust and acetic acid gave dideoxycycloheximide (V) (m.p. 135–136°).

Anal.—Calcd. for $C_{15}H_{25}NO_2$: C, 71.67; H, 10.0; N, 5.57. Found: C, 71.90; H, 9.84; N, 5.76.

Determination of the optical rotation of V gave $[\alpha]_D^{25}$ −9.07° (c = 6% in acetone).

The observation that V is optically active establishes that the methyl groups are *trans*, since the corresponding compound with *cis* methyl groups would be *meso* due to its plane of symmetry.[1] Therefore, the absolute configuration of both methyl groups of cycloheximide is correctly depicted in I. Similar conclusions have recently been obtained by a totally different procedure (3).

(1) Eisenbraun, E. J., Osiecki, J., and Djerassi, C., *J. Am. Chem. Soc.*, **80**, 1261(1958).
(2) Kornfeld, E. C., Jones, R. G., and Park, T. V., *ibid.*, **71**, 150(1949).
(3) Lawes, B. C., Abstracts of the 139th meeting, American Chemical Society, St. Louis, Mo., March 1961, p. 33N.

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[1] The fact that there was no change in configuration of the methyl groups in this reaction sequence and the description of other stereochemical interrelationships will be the subjects of a future paper.

U.S.P. XVI Monographs for Radiopharmaceuticals

Sir:

The January 1961 issue of THIS JOURNAL published a letter criticizing the U.S.P. XVI monographs for radiopharmaceuticals. The author of that letter, C. T. Peng, perhaps lacks a complete understanding of the basic principles that govern the U.S.P. revision program, one of which requires that revisions of the monographs be supported by well-documented experimental data. Dr. Peng has criticized certain aspects of the monographs, but the data on which his criticisms are based are not readily available to the scientific community or even to the U.S.P. Revision Committee. On behalf of this Committee and others who donated time and effort in helping to prepare the monographs for radioactive drugs, I should like to answer some of Dr. Peng's criticisms and confirm the suitability of the monographs for compendial purposes.

Dr. Peng made six specific criticisms in his letter that may be summarized briefly as follows:

1. The effect of radiolysis upon the added bacteriostatic agents was not considered and the effect of such radiolysis is unknown.

2. As a result of radiolysis, some of the iodide in sodium radio-iodide solution U.S.P. is organically bound and unavailable for therapeutic use.

3. The presence of heavy metals in sodium radiophosphate solution U.S.P. results in loss of activity.

4. The *Identification* test for sodium radiophosphate solution U.S.P. is not suitable, and there is a marked lack of consistency in some of the various calculations.

5. The *Assay* procedures are not suitable.

6. The U.S.P. is deficient in failing to specify a definite counting instrument and to provide specific information regarding counting techniques.

We should like to discuss each of the six points mentioned above separately.

Point 1. In reference to the radiolysis of added bacteriostatic agents, Dr. Peng claims that "the presence of odoriferous compounds and the discoloration in these preparations, as noted in the U.S.P., may be explained on this basis." He also states: "Neither the toxicity to man of these radiation decomposition products nor their value as bacteriostatic agents have been determined; indeed the extent of radiation decomposition of the added bacteriostatic agent in the preparation has not been evaluated."

The bacteriostatic agents commonly used for these products have odors of their own and, when added to the radioactive solutions, impart some of their odor to the solutions. For this reason the U.S.P. monographs make provision for some odor in the solutions and not, as Dr. Peng conjectures, to provide for radiolysis products. Similarly, the provision made for darkening is based upon the fact that it is characteristic of both the solution and the glass of the container to darken on standing.

We agree that data in regard to the possible toxicity of radiation-decomposed bacteriostatic agents may be lacking, but, insofar as we are aware, no evidence exists that such decomposition takes place. Dr. Peng was not correct in stating that no evaluation has been made of the extent of radiation decomposition of added bacteriostatic agents for we understand that at least one of the producers has made such an evaluation. When activity concentrations similar to those present in therapeutic preparations and longer-than-usual holding periods were used, both ultraviolet spectroscopy and gas chromatographic determinations showed the presence of the same quantities of the same organic materials in the solutions exposed to radioactivity as in those not exposed to such radioactivity. It thus appears that radiolysis of added bacteriostatic agents is not a factor of importance for these products.

Point 2. If Dr. Peng is correct in his assumption that some of the radioactive iodine is bound by the bacteriostatic agent, this may indeed be the consequence of radioactivity in the solution. No explanation for Dr. Peng's unpublished observations can be offered, but it would be helpful to know whether the solutions used in his experiments conformed to the U.S.P. XVI test for *Radiochemical purity*. An amount of organically bound iodide sufficient to give the response reported by Dr. Peng should be detectable by this procedure, and, if it is not so detectable, consideration should be given to a revision of the monograph.

Point 3. The effects of heavy metals on both the adsorption and colloid formation characteristics of phosphorus[32] were recognized during the preparation of the monograph for this product, and this is the reason for the statement in the monograph: "Inactive sodium phosphate carrier may be added during processing." Although most of the sodium radiophosphate solution U.S.P. available commercially contains added carrier, we were informed that some solutions do not contain such carrier. This illustrates one of the difficulties encountered in writing a monograph

for a product available from several suppliers. If the monograph specifies that carrier must be added, then the equally acceptable carrier-free product would not conform to the monograph specifications. Our solution in this instance was to make addition of carrier optional.

Point 4. We do not agree with Dr. Peng's opinion that a maximum range determination should be used to identify phosphorus[32]. Although it is obvious that more than one procedure may be suitable for identification purposes, the persons consulted in this regard recommended adoption of the absorption coefficient procedure because of its relative simplicity and suitability for routine use. Maximum range determinations are complicated, and we are informed that they do not lend themselves to routine use. In this regard one of our advisors has written: "Most investigators . . . feel it is much more accurate to compare the absorption under a given set of conditions and detector with the absorption of a sample of known purity. In this manner the various factors referred to (in the Peng letter) are eliminated in the comparison method."

(It should be mentioned at this time that the First Supplement to U.S.P. XVI will revise this *Identification* test procedure to specify the use of one additional absorber of 800-mg.-per-square-centimeter thickness.)

The apparent lack of conformity of the similar calculations is not a major oversight although we agree that conformity would be preferable. The factor of 100 in the I^{131} and P^{32} calculations is required to convert the results obtained on the specified 0.01 ml. of sample to a mg.-per-ml. basis. In the other procedures the sample size is not specified; hence, the inclusion of such a factor is unnecessary.

Point 5. Insofar as we are aware, the use of calibrated standards has not caused undue difficulties. Although the so-called permanent standards are undoubtedly used by some laboratories, our advisors recommended that we omit specification of them in the U.S.P. monographs. Results obtained using permanent standards during the preparation of the I^{131} and P^{32} monographs showed some discrepancies that could not be explained, and this supported the recommendations of our advisors. Moreover, permanent standards were available only for I^{131} and P^{32} when the monographs were prepared; thus it was impossible to consider such standards for the other radiopharmaceuticals.

Point 6. The suggestions to specify the use of a particular counting instrument and to include detailed instructions on the application of counting techniques show a definite lack of understanding of U.S.P. policies. It is an established policy of the U.S.P. to refrain from specifying use of a particular kind or make of instrument when several different instruments are suitable.

The U.S.P. is not designed to be a textbook on the various methods of analysis included therein. U.S.P. procedures will presumably be used by qualified personnel having knowledge of the instruments and operations required, and for this reason detailed discussions of the various analytical techniques are not included in the U.S.P.

One additional point may be worth mentioning. In the last paragraph of his letter, Dr. Peng implies that bacteriostatic agents are added to sterilize the solutions. Addition of bacteriostatic agents is not a substitute for proper sterilization of the solutions during manufacture. The bacteriostatic agents are added to maintain sterility during storage and use, but only after the solutions are sterilized by conventional means.

In conclusion, I should like to emphasize that each of the points raised by Dr. Peng will be considered further by a panel of experts on radioactive drugs in connection with review of the U.S.P. XVI monographs for U.S.P. XVII. Serious consideration can be given, of course, only to published data or to the unpublished but well-documented data submitted to us by various investigators. All such data should be sent to Dr. Lloyd C. Miller, Director of Revision, United States Pharmacopeia, 46 Park Avenue, New York 16, N. Y., for reference to the U.S.P. Subcommittee concerned.

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